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NEW ZEALAND JOURNAL OF SCIENCE

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PRIMARY PRODUCTION IN A NEW ZEALAND WEST COAST PHYTOPLANKTON BLOOM

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Summary

Dense phytoplankton blooms, frequently visible to the unaided eye, are of common occurrence in the waters off many west coast beaches in the North Island of New Zealand, particularly in the winter months. The dominant species is *Chaetoceros armatum* (?) (probably a new species) while *Asterionella japonica* may be abundant at times. These blooms support a large biomass of benthic and nektonic species, but zooplankton is relatively scarce.

Daily samples at 0930 were taken from a point near the entrance to Waitarere beach from 11 to 21 August 1959. Measurements were made of gross primary production by the light and dark bottle oxygen method with light-bath incubation, and of plant pigments by spectrophotometry of 90% acetone extracts. Quantitative counts were made of phytoplankton.

Gross production is estimated as ranging from 20 to 400 mg C/hr/m^3 at 10°c and 300 foot candles. Production is correlated to chlorophyll *a* content ($r = 0{\cdot}92$) and to number of cells of *Chaetoceros armatum* (?) ($r = 0{\cdot}87$). 3–3·5 mg C per hour is utilised per mg chlorophyll *a*, and (very approximately) 1 mg C per hour per 10^6 *Chaetoceros* cells. Where production is high the rate of increase in oxygen concentration decreases with time, so that the shortest incubation periods (2 hr) are considered the most reliable estimates. This decrease is probably due to modification of the environment in the bottle by photosynthesis, since pH changes of up to 0·2 have been observed.

It is considered that the blooms studied are two orders of magnitude less than the maximum that may be reached on this beach, so that the gross instantaneous rate of primary production must at times reach an exceptionally high figure. However, owing to the scarcity of zooplankton predators and the high mortality rate of phytoplankton by stranding, it is likely that plant nutrients are recycled relatively rapidly, so that net production of organic material on an annual basis is not unduly great. It is also possible that environmental changes similar to those observed in the bottles may provide an upper limit to the daily photosynthetic activity of the denser blooms.

INTRODUCTION

The prevalence of dense phytoplankton blooms on certain New Zealand, North Island west-coast beaches is well known and has been recorded by Rapson (1954) and Cassie (1955) in studies of the toheroa, *Amphidesma ventricosum,* a filter-feeding lamellibranch which appears to be associated with these blooms. Rapson estimates the numerical abundance of diatom cells as reaching a maximum of 10^5 per ml. Phytoplankton is frequently dense enough to be plainly visible as a green or brownish coloration in the surf, and is at times deposited on the beach by the receding tide as a scum up to an inch in thickness. The appearance at the edge of the surf of a typical bloom is illustrated in Fig. 2. Although the photograph was taken at Muriwai, it represents equally well the conditions which sometimes prevail at Waitarere. The vast volume of organic matter represented is undoubtedly associated with a rich source of mineral nutrients which probably originates from upwelling water. A water sample taken from Muriwai beach, North Auckland (see Fig. 1) has been examined by the New Zealand Institute of Nuclear Sciences and found to be depleted in ^{14}C by $2{\cdot}0 \pm 0{\cdot}4\%$ with respect to contemporary *Pinus* wood. While the significance of this information is not entirely clear, it would suggest that the upwelling water has been out of contact with the atmosphere for about 250 years, thus indicating an upwelling of relatively deep origin.

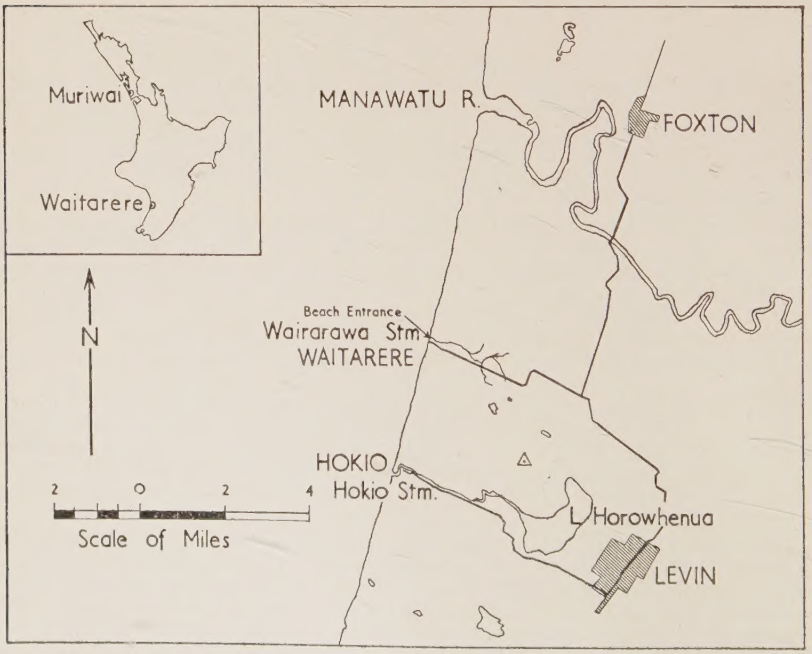

FIG. 1—Locality map showing Waitarere beach.

FIG. 2—Muriwai beach showing a bloom of *Chaetoceros armatum* (?) as dark patches on the sand and the surface of the water. White objects on the sand are dead shells, principally *Amphidesma subtriangulatum* and *Spisula aequilateralis*.

Although a considerable number of diatom species has been recorded in the phytoplankton of these coasts, at times of most intense blooming, one species – *Chaetoceros armatum* (?)* – is almost invariably dominant, sometimes to such a degree that the flora is virtually monospecific. The New Zealand distribution of this species appears to be restricted to the waters adjacent to exposed surf beaches particularly on the west coast. Though present on New Zealand west-coast beaches during all months of the year this species is most abundant in winter when it frequently forms visible blooms in the water near all beaches containing toheroa. Indeed the presence or absence of abundant *C. armatum* (?) would appear to be a limiting factor in the distribution of this mollusc. A second diatom, *Asterionella japonica,* is also frequently abundant and sometimes dominant, though probably never forming as great a biomass as *C. armatum* (?).

*The specific name of this diatom is queried, since its identity is in some doubt. *C. armatum* was originally described by West (1860) from material obtained on the Norfolk coast in 1857, and was subsequently found abundant on various other parts of the coastline of the British Isles. Wood *et al.* (1959) and Rapson (1954) both identify as *C. armatum* a species from New Zealand which is undoubtedly identical with that discussed in the present paper. However, Dr N. I. Hendey (pers. comm.) has recently examined some New Zealand material and reports that this is almost certainly a new species, or at least a new seasonal or geographic form. He states that *C. armatum* is now seldom, if ever, found near its type locality, but occurs frequently in many parts of the Pacific, often in dense monospecific blooms, similar to those found on New Zealand beaches.

The phytoplankton population provides food (directly or indirectly) for a variety of benthic species. On Waitarere Beach (Fig. 1) where the present investigation was carried out, the toheroa occupies a band of beach midway between low and high tide marks, and possibly also some part of the sublittoral zone (Cassie 1955). In the intertidal zone are also to be found buried in the sand the polychaete *Aglaophamus macrura,* and the sand-shrimp *Callianassa filholi.* The stomatopod *Lysiosquilla spinosa* is probably also present, although definite records are not available for Waitarere. An aqualung search showed the dominant macroorganisms to be the tuatua (*Amphidesma subtriangulatum*) from low tide to about 2 metres depth, and beyond this, the biscuit urchin (*Arachnoides zelandiae*) down to 5 metres depth and about 200 metres beyond low tide level which was the limit of the search.

OBJECTS OF INVESTIGATION

The purpose of the present investigation was two-fold. Firstly, it will give a measure of the potential for primary production of organic matter in this region and of the day-to-day fluctuations in abundance and species composition of the phytoplankton over a short period. Secondly it serves as a pilot experiment determining the suitability of this flora for a proposed study of the relationship between primary production and the metabolic processes of the phytoplankton. Many studies of this type in the field are handicapped by the relatively small number of organisms per unit volume, and by the specific complexity of the flora under natural conditions. Thus *in vitro* measurements of photosynthesis and respiration must be made close to the lower limit of sensitivity of the available techniques, or after undesirably long exposure of the sample to artificial conditions in the holding vessel. Interpretation of the resulting data is confused owing to the difficulty of assigning appropriate weighting factors for the contributions of the different species concerned. Some of these objections may be overcome by the use of concentrated monospecific cultures in the laboratory, but conclusions from such methods will not necessarily be applicable to natural populations.

C. armatum (?) provides a population which is at times both virtually monospecific and sufficiently abundant for the oxygen produced by photosynthesis or removed by respiration to be readily measured within a relatively short period of time. Thus there is some possibility of conducting a field investigation with many of the desirable features of a laboratory experiment. While the population is in many respects an abnormal one, and cannot be considered as representing "typical" oceanic plankton, it is at least being studied in its natural state rather than under the highly artificial conditions of a laboratory culture. While ideal conditions were not completely realised in the present experiment, the results are sufficiently informative to pave the way for future investigation.

METHODS

Sea water and plankton samples were taken from the beach at a point close to the beach entrance (see Fig. 1) between 0900 hr and 0930 hr each day from 11 to 21 August 1959, and also at 2130 hr on 17 August. The

collecting procedure was for one person to wade out waist deep in the surf and fill two plastic buckets with sea water. By the time these were returned to the beach, most of the suspended sand had settled. The water temperature was recorded, and water samples were taken by immersing "light" and "dark" bottles completely in one of the buckets and screwing on the cap while still under water. The bottles were placed in the dark immediately after filling. A winchester was also filled with seawater for phytoplankton counts, pigment extraction, and salinity determinations. While a second person was taking these samples from the bucket, a small tow net of No. 25 nylon bolting cloth was towed in the surf for about five minutes and the resulting sample preserved in 5% neutral formalin. Light and dark bottles were returned to a laboratory (which was set up close to the sampling point) and placed in the light incubator at 0930 hr.

Both for plankton and pigment determinations the contents of the winchester were thoroughly shaken before the required volume of water was drawn for centrifuging. However, on the morning of 17 August, a large quantity of suspended organic matter was present, making plankton counts almost impossible. On this occasion the water in the winchester was allowed to settle for 30 minutes before drawing the plankton sample. A pigment extraction was made from the water both before and after settling.

Phytoplankton

A 100 ml water sample was concentrated with a Foerst continuous centrifuge and counted in a modified Sedgwick-Rafter cell into which the entire sample was placed. A fraction of the cell varying from 1/100 to $\frac{1}{8}$ of the area (depending on the density of organisms) was counted, using a phase contrast 16 × objective and 8 × eyepiece. Difficulty was experienced in counting on some occasions owing to the abundance of suspended detritus, so that the estimates of cell numbers are probably the least accurate of the determinations made in this series. Net samples were also examined to give a more detailed picture of the specific composition of the plankton.

Pigment Analysis

A litre sample was centrifuged, desiccated under reduced pressure, and extracted for 24 hours in 10 ml of 90% aqueous acetone. The extract was stored in a refrigerator in the dark and submitted (within a week in each case) to the Dominion Laboratory, D.S.I.R., Wellington, where a spectrophotometric determination of pigments was made, using the method described by Richards and Thompson (1952). Re-checking of some of the determinations showed that no significant change took place in the pigment during storage.

Primary Production

Measurement of primary production was based on the light and dark bottle oxygen technique as originally described by Gaarder and Gran (1927). Dark bottles were constructed by wrapping adhesive black P.V.C. tape around 12 oz (300 ml) jars which were provided with black plastic lids. These were paired with clear bottles which had white translucent plastic lids.

Six pairs of bottles were filled each day, thus enabling three sets of duplicate dissolved oxygen determinations to be made after incubation for three different periods. Two additional bottles were also collected for determination of initial oxygen concentration. The six paired bottles were stacked in groups of four, forming three tiers, in the centre of a perspex tank 30 cm square and 50 cm deep, filled with sea water. The tank was enclosed by a white painted wooden box 53 cm square and 61 cm deep with a 20 watt fluorescent light arranged vertically on each of the four sides, providing an illumination of 300 foot candles (approximately 3,000 lux) on the bottles. The light intensity could have been increased by a factor of 3 or 4 with some advantage, but since no method of temperature control was available, the heating effect would have been excessive. Although the fluorescent tube itself is a cold-light source, there is inevitably a certain amount of heat generated in the usual commercial fittings.

Two pairs of bottles were removed at 2, 4, and 8 hours after incubation commenced. The water was carefully siphoned to 250 ml reagent bottles and the dissolved oxygen content determined by the standard Winkler titration. On some occasions pH determinations were also made, using glass and calomel electrodes and a Cambridge pH meter. A record was kept of the temperature of the water in the perspex tank at commencement of incubation and at the times of removal of bottles.

RESULTS

For convenience of reference, the date on which a sample was taken will also be employed as the sample number.*

Phytoplankton

Quantitative estimates of all phytoplankton species are given in table 1. Counts are reported as cells per ml (which may be alternatively read as 10^6 cells per cubic metre). Where a species is recorded from the net sample only a coded estimate of its abundance is given, ranging from "r" (one only observed) to "+++" (common) (see key at foot of table 1). In general the net estimates were consistent with those from the centrifuge, although, as might be expected, the net invariably yielded more species. For the two most abundant species, *Asterionella japonica* and *Chaetoceros armatum* (?) the mean number of cells per chain is recorded in brackets below the estimate of abundance.

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*On 17 Aug. four samples were taken. These are designated as follows:

17: Routine daily sample at 0930 hr (note also that in table 2 this sample provides two different pigment estimates, the estimate which departs from routine procedure being marked with an asterisk).

17 (p.m.): A sample following the usual routine, but taken at 2130 hr.

17A, 17B: Two additional samples not submitted to the usual routine. These are omitted from table 1, but are included in table 3 and in the discussion of pigments.

Pigments

The estimated quantities of plant pigments are given in table 2. Chlorophylls *a* and *b* are reported in milligrams, and chlorophyll *c* and carotenoids in specified pigment units (MSPU) as defined by Richards and Thompson (1952). One MSPU is approximately one milligram, although its exact weight is not known owing to uncertainty as to the molecular formula of the pigments concerned. On 17 August two additional samples were taken from a *C. armatum* (?) bloom about five miles north of the beach entrance. 17A was taken from the scum deposited on the sand, and 17B was scooped with a sheet of paper from the foam at the surf edge. Both samples were virtually monospecific, so that they provide the best available estimate of the pigment composition of this species. Since a quantitative estimate for them would be inappropriate, they are omitted from table 2, but the relative proportions of the pigments are given at the bottom of table 3. It will be noted that some of the estimates for carotenoids have small negative values, but that these are within the limits of the expected instrumental error of the method as given by Richards and Thompson.

Primary Production

Results of light and dark bottle determinations are given in table 4, oxygen content being expressed in millimetres per litre of water at N.T.P. Initial oxygen concentrations have been omitted from the table since in most cases they do not differ from the two hour dark bottle concentration by any more than the error of the determination. In a few instances it is also suspected that errors of technique have invalidated the determination. However, the general values and significance of the initial oxygen concentration will be referred to further in the discussion. In every case the water at 0930 hr was found to be slightly supersaturated, usually by about 5%.

Except where one of the replicates was discarded (owing to the presence of gas bubbles, etc.) the difference between light and dark bottles is a difference between two means, and thus may be followed by the standard error of this difference. The mean standard error for all differences is 0·10 ml/l, which is about double the error to be expected from the Winkler titration. (Since the error of a single titration is about 0·05 ml/1 the error of the difference between the means of two pairs will be the same). The additional error is probably attributable to differences between initial oxygen concentration of different bottles, while in some instances suspended organic matter may have also contributed to the uncertainty of the titration. With respect to differences in initial oxygen content, it is evident that accuracy could be improved appreciably if all determinations could be made on one bottle, as in the polarographic method described by Carritt and Kanwisher (1959) and Kanwisher (1959).

TABLE 1—Phytoplankton, Estimated Cells per Millilitre

Species	Date (August 1959)												Total	Samples
	11	12	13	14	15	16	17	17(pm)	18	19	20	21		
Phytoplankton, 100 ml samples and nets														
Asterionella japonica	24	12	58	40	320	99	1,700	1,700	94	220	180	2,200	6,647	12
	*(5·6)	(5·8)	(5·8)	(5·6)	(12·4)	(9·2)	(11·5)	(14·8)	(12·2)	(12·2)	(14·8)	(12·0)		
Chaetoceros armatum (?)			330	18	54	170	78	63	4	7	17	3	744	10
			*(3·4)	(3·5)	(3·6)	(3·6)	(3·7)	(4·0)	(5·7)	(5·7)	(5·8)			
Lauderia annulata	0·6		0·8	1·0		3·2	4·1	12·2	2·1	1·5	4·6	25·8	56	10
Grammatophora sp.	4·6	1·9	++	2·0	25·5	2·4				+	0·5		37	6 + 2
Cerataulina pelagica			0·3	0·3	0·3	3·7	2·0	2·7	1·2	3·2	5·8	10·6	30	10
Navicula lyra	0·8	0·5	0·7	0·5	0·5	0·5	0·3			0·1	0·1	2·9	6·9	10
Rhizosolenia imbricata				+	+	1·5	2·7	++	0·3	0·4	0·7		5·6	5 + 3
Thalassiothrix nitzschiodes	0·3	0·2	0·5	0·7	0·7	0·3		+			2·7		5·4	7 + 1
Unarmoured dinoflagellate			3·4	1·2	+	0·5	+		+				5·1	3 + 3
Eucampia zoodiacus						r	2·0	2·0		r			4·0	2 + 2
Rhizosolenia setigera	0·4					r	0·3	+	+	++	0·2	1·9	2·8	4 + 4
Pleurosigma sp.	0·1		0·3	r	0·3	0·3				+		1·0	2·0	5 + 2
Nitzschia closterium	1·0	0·3			+	0·2	+		+	0·1			1·6	4 + 3
Thalassiosira decipiens	0·3	0·3	+		0·2	0·5							1·3	4 + 1
Rhizosolenia styliformis		+	+	+	+			+	+++	++	0·2	1·0	1·2	2 + 7
Melosira granulata	1·0												1·0	1
Scenedesmus sp.	0·5		0·3										0·8	2
Thalassiosira hyalina							0·7	+	+	0·1	r		0·8	2 + 3
Biddulphia aurita								0·7	+	+			0·7	1 + 2
Gymnodinium sp.		0·2	0·5										0·7	2

* Figures in brackets = mean number of cells per chain.

Thalassiosira condensata				0·7									0·7	1
Melosira sulcata		0·5											0·5	1
Prorocentrum micans	0·1	0·2	0·2										0·5	3
Aulacodiscus kittoni	0·1	0·1	0·2	++	r	┐			r	┤		┐	0·4	3 + 6
Amphora sp.						0·3							0·3	1
Chaetoceros secundum					r					0·3			0·3	1 + 1
Nitzschia seriata									0·3	r			0·3	1 + 1
Peridinium conicum										r	0·3		0·3	1 + 1
Radiolaria sp.		r					0·3						0·3	1 + 1
Biddulphia mobiliensis		0·1			r				0·1	0·1		r	0·3	3 + 2
Coscinodiscus centralis		0·2		+	r				┤		+		0·2	1 + 4
Streptotheca thamensis			0·2										0·2	1
Amphiprora sp.				0·2									0·2	1
Navicula sp.					┬	0·2							0·2	1 + 2
Synedra frauenfeldii					0·2	r		+					0·2	1 + 2
Oscillatoria sp.	0·2												0·2	1
Distephanum speculum			0·2		r	r							0·2	1 + 2
Asterionella formosa		0·1		┤					0·1				0·1	1 + 1
Tintinnoinea (sp. 1)													0·1	1

Scale of Abundance for Species found in net samples only

r = one specimen only
\+ = scarce
++ = occasional
┤ ++ ─ common

TABLE 1—*continued*—Less Common Species

Species	11	12	13	14	15	16	17	17(pm)	18	19	20	21	Samples
Total cells per ml ..	34	17	396	65	402	283	1,790	1,781	102	233	212	2,246	
Total species (omitting nets)	14	13	14	10	9	14	10	6	8	10	11	8	
Cells counted (raw data) ..	404	197	2,354	383	2,357	1,651	5,189	2,668	1,206	2,711	1,374	2,328	
α = Index of diversity	2·81	3·13	1·98	1·88	1·18	2·10	1·19	0·73	1·15	1·31	1·63	1·04	
$s\alpha$ = Standard error of α	0·35	0·46	0·19	0·26	0·14	0·21	0·23	0·11	0·15	0·14	0·19	0·13	
Phytoplankton, Net samples only													
Actinoptychus adriaticus			+						+	+		+	5
Chaetoceros didymum						r			+				2
Coscinodiscus concinnus		+								r			2
Ditylum brightwellii									r	r			2
Asteromphalus hookeri			+										1
Biddulphia chinensis									+				1
Chaetoceros compressum					r								1
Chaetoceros concavicorne		+											1
Chaetoceros criophilum										r			1
Corethron criophilum									+				1
Coscinodiscus marginatus											r		1
Planktoniella florea									r				1
Rhizosolenia stolterfothii										r			1
Triceratium alternans									r				1
Diploneis crabro							r						1
Stenopterobia intermedia						r							1
Ceratium pentagonum										r			1
Tintinnoinea (sp. 2)				r									1
Total species (all samples)	14	17	19	17	20	21	13	12	23	25	15	12	
Zooplankton													
Copepod nauplii						+			0·2	+	++		1 + 3
Adult copepods									+				0 + 1
Cladocera (*Polydora polybranchiata*)								+					0 + 1
Cladocera						+							0 + 1

DISCUSSION

Plankton

In all, 57 species of phytoplankton were found, comprising 46 diatoms, 8 dinoflagellates, 2 tintinnids and 1 radiolarian (table 1). Of these, only 39 species were counted in the 100 ml samples, the remainder being observed in the net samples, where a very much larger volume of water was filtered. No attempt has been made to estimate microflagellates or other smaller and more delicate photosynthetic organisms of the nannoplankton. The tentative assumption has been made that, in the more intense diatom blooms, these species will not contribute significantly to production. Since production is highly correlated with the numbers of the most abundant diatom, this assumption seems to have some justification, though obviously a more critical assessment will be necessary in future work.

Zooplankton was particularly scarce, only 3 or 4 species being found, and only one of these in the 100 ml samples. Further sampling will be necessary to establish whether this is the usual abundance level of zooplankton in the region. However, the present information is consistent with the findings of other workers, such as Hardy (Hardy and Gunther, 1935), who based his "animal exclusion" hypothesis on the finding that, where phytoplankton is particularly abundant, zooplankton is often scarce. If the observed proportion of animal to plant plankton is maintained on Waitarere beach for any length of time, it would appear that the usual food chains, where small crustacea are assumed to be the principal grazing animals, do not apply in this region. While large numbers of diatoms are undoubtedly consumed directly by *Amphidesma* and other filter feeders, possibly a similar or even greater number die through being cast up on the beach. Some of the dead material would probably enter the diet of the detritus-feeding *Arachnoides* (biscuit urchins), but it is likely that the majority would be decomposed by bacteria, so that plant nutrients would be returned fairly rapidly to the water and thus be available for a fresh cycle of production far sooner than if they had remained "locked up" in the tissues of a relatively long-lived animal. If this is the case, a relatively high instantaneous rate of primary production may be maintained by recycling of the same nutrients, without the necessity for their rapid renewal from upwelling or other external sources.

In order to obtain a comparative measure of the specific diversity of the populations represented, the statistic α has been computed for each sample (table 1). α, or the index of diversity, is a parameter developed by Fisher (Fisher, Corbet and Williams, 1943) and applied by Williams (1944) to various terrestrial plant and animal populations. Taylor (1953) has used the same concept for demersal fish catches but it does not appear to have been previously employed for pelagic populations. It is assumed that, in a sample from a population, the number of species represented by 1, 2, 3, 4, etc. individuals may be described by the logarithmetic series:

$$n_1,\ n_1x/2,\ n_1x^2/3,\ n_1x^3/4 \ldots\ldots\ldots \text{etc.}$$

(n_1 is the number of species represented by one individual only, and x is

a constant less than unity). As the size of the sample increases, n_1 tends toward a constant value x, where:

$$x = N/ (N + \alpha)$$

(N is the total number of individuals of all species in the sample.) Since x is independent of sample size it may be employed as an index of the specific diversity of the population. Fisher also gives a procedure for computing the standard error of α from which tests of significance of difference between samples may be made.

It will be seen from table 1 that α* has a range between about 1 and 3, a relatively low figure compared with that recorded for most terrestrial populations (Williams 1944), but probably fairly representative for plankton populations, which tend to contain a large number of individuals relative to the number of species. The general pattern of change is obscure, although there is a fairly consistent lowering of the index from the beginning to the end of the period. There is clearly some heterogeneity, suggesting that several distinct populations are represented, perhaps because changes in the flora are partly due to the flow of water, and hence of different plankton populations, past the point of sampling. On the other hand, there is some tendency for the index to fall at the onset of a bloom. This could be explained on the assumption that "blooming" is confined to one or a few species, without any parallel increase in the numbers of rarer species. Thus a single population of phytoplankton which remained perfectly discrete and continuous in the biological sense might well change within a few days to a different statistical population as judged by the index of diversity. It would appear that, in the present context, the true meaning of α is open to some doubt and requires further investigation.

In table 1 the estimates for the two most abundant species, *Asterionella japonica* and *Chaetoceros armatum* (?) are accompanied by figures in brackets which are the mean number of cells per chain. In both species, this number tends to be greater toward the end of the sampling period, the greatest change being between 14 and 15 August for *Asterionella* and between 17 and 18 August for *Chaetoceros*. Since neither of these changes corresponds with any other pronounced feature of the population cycle, it is not possible to attribute any particular significance to them at present.

Pigments

The significance of chlorophyll *a* as an estimate of primary production potential will be discussed in a later section. Examining the detailed composition in table 2 it is seen the most abundant pigments are chlorophylls *a* and *c* which are present in about equal quantities, while chlorophyll *b* and non-astacin-type carotenoids (i.e. the plant carotenes) are relatively scarce.

*In computing α, it is necessary to return to the raw data since the number of species found will be dependent on the actual proportion of the sample counted. For this purpose the number of cells counted is given in table 1, as well as the estimated number of cells per ml.

This is not entirely consistent with the usual pigment composition of diatom populations, since Fogg (1953) indicates that the dominant pigments are chlorophyll *a* and β-carotene, with chlorophyll *c* comprising only a small fraction of the total and chlorophyll *b* absent. While the empirical method of assay which has been employed is a well-established one, it would seem that estimates of the minor pigments should be treated with some caution, particularly in view of the somewhat unusual characteristics of *Chaetoceros armatum* (?). As might be expected from the scarce zooplankton, astacin-type carotenoids are negligible in quantity.

TABLE 2—Pigment Composition

Date: August	Chlorophylls/m³			Carotenoids/m³	
	a mg	*b* mg	*c* MSPU†	astacin MSPU†	non-astacin MSPU†
11	7	1	8	1	2
12	11	3	17	2	2
13	46	3	20	0	15
14	6	1	11	1	1
15	7	1	5	2	− 1
16	16	4	20	2	1
17*	14	3	23	2	4
17	91	13	93	6	13
17 (2130)	46	8	58	3	5
18	6	1	11	1	1
19	8	2	15	1	1
20	9	2	10	1	1
21	4	1	16	5	0

*Water sample allowed to settle for 30 min. before centrifuging.

†The MSPU is an arbitrary pigment unit with a value of approximately 1 mg.

In order to give some estimate of the relationship between pigment and species composition, table 3 shows the relative proportions of pigments, on the basis that chlorophyll *a* = unity, for six selected samples. Samples 17A and 17B, as already discussed, are virtually pure cultures of *Chaetoceros armatum* (?), while in 13 the same species is dominant. Samples 11 and 12 are predominantly *Asterionella*, with *Chaetoceros* absent and other species negligible in amount, although it is possible that there is some contamination with pigments from dead organic matter. Sample 21 is also predominantly *Asterionella*, though other species are not entirely negligible. Bearing in mind the relative validity of the various samples, a clear distinction between the two dominant species may be detected from the chlorophyll *c* : *a* ratio, which in *Asterionella* is greater and in *Chaetoceros* less than unity. The spectral composition of pigments was reflected to some extent by the visible blooms in the water, *Asterionella* tending to brown and *Chaetoceros* to green in colour. Some amplification of the above information may have a limited use in the characterisation of blooms. Thus it would provide a rough measure of the relative proportions of the species in a flora in which these two alone are dominant, but it is doubtful if the pigment ratios are sufficiently constant

to be of general use. It is not possible, for example, to coordinate them on a precise quantitative basis with the various specific combinations found in the present experiment.

TABLE 3—Pigment Composition Relative to Chlorophylls

Sample	Chlorophylls *a*	*b*	*c*	Carotenoids astacin	non-astacin	Dominant Species
11	1	0.14	1.15	0.14	0.29	*Asterionella japonica*
12	1	0.27	1.55	0.18	0.18	,,
21	1	0.25	4.00	1.25	0.00	,,
13	1	0.06	0.44	0.00	0.33	*Chaetoceros armatum* (?)
17A	1	0.02	0.15	—0.02	0.30	,,
17B	1	0.02	0.17	—0.02	0.31	,,

Primary Production

Using the light and dark bottle technique, the following three quantities may be obtained in any one set of determinations:

I = Initial oxygen content of water before incubation.
L = Oxygen content of light bottle after incubation.
D = Oxygen content of dark bottle after incubation.

From these, the following estimates are available:

Gross production = total oxygen produced by photosynthesis = L–D
Respiration = total oxygen consumed by respiration = I–D
Net production = gross production – respiration = L–I

It is assumed that respiration proceeds at the same rate in both light and dark conditions.

In the present investigation (table 4), the initial oxygen estimate, I, has been discarded, since the difference, I–D, is too small in relation to its error to give a valid estimate of day to day changes in respiration rate. However, respiration was in all cases less than 10% of total photosynthesis. It has been suggested by Ketchum *et al.* (1958) that in a "healthy" growing population of phytoplankton, net production will be 90–95% of gross production, so that by this criterion the diatom populations studied were in an actively growing stage and not appreciably limited by nutrients or other factors. Initial oxygen content always showed slight supersaturation, usually by about 5%. This might be expected since samples had been taken close to the time of maximum photosynthesis, and after exposure of the ocean water to several hours of daylight.

For convenience of comparison with other published data, oxygen differences are converted to their equivalent in milligrams of glucose carbon per m^3 per hour, by applying the conversion factor, 268·2 which is derived as follows:

Conversion factor $= 1000dC/bO_2$
where d = normal density of oxygen = 1·42896
b = time of incubation in hr = 2, 4, or 8
C = atomic weight of carbon = 12·011
O_2 = molecular weight of oxygen = 32
1000 = conversion from litres to cubic metres.

In converting to glucose carbon, it is assumed that photosynthesis may be described by the equation

$$6\,CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

in which oxygen evolved is the molar equivalent of carbon dioxide consumed. While there is good reason for believing that this relationship does not hold exactly, and may vary under different circumstances, it is a sufficient approximation for present purposes, since only order-of-magnitude comparison can be made with other published data.

It is clear from table 4 that the estimate of gross production varies considerably between the different incubation times in any one sample. Comparing adjacent estimates (i.e. 2 and 4 hr; 4 and 8 hr) about half the differences are significant at the ($P = 0·05$) level (as indicated by asterisks in table 4). There is in most cases a downward trend in production with time, which is most pronounced on 13 and 17 Aug. which are the days when blooming was at its peak. Among the intermediate rates of production a similar falling off is seen on 17 (p.m.) and 21 Aug. and for 2–4 hrs on 15 Aug., but not for 4–8 hrs on 15 Aug. Of the remaining significant differences, that between 2 and 4 hrs on 19 Aug. also shows a departure from the downward trend, although here production rates are low and probably more subject to errors of estimation.

While the discrepancies must be noted, it would seem reasonable to conclude from the data that there is a real falling off with time in the rate of oxygen evolution particularly for the higher rates of production. This would seem to be at variance with the statement by Ryther (1956b) that "photosynthesis in samples enclosed in glass bottles may proceed at a constant rate for 24 to 48 hours, after which it invariably decreases". However, there is little doubt that Ryther was in this instance concerned with relatively low levels of production, where photosynthesis would be unlikely to induce significant changes in the environment. In the two blooms on 13 and 17 August, it is clear that the chemical properties of the environment have been appreciably modified. On 17 August the initial pH was found to have increased in the light bottle from 8·25 to 8·45 in 8 hours, a change which would undoubtedly have an adverse effect upon the rate of photosynthesis.

Considering the diminution of photosynthesis rate on the quantitative basis and averaging the figures for 13 and 17 August (which are roughly similar) it is found that the mean rate of oxygen evolution fell from 0·67 ml/l/hr between 0 and 2 hours to 0·125 ml/l/hr between 4 and 8 hours, the second figure being approximately 20% of the first. Thus the photosynthesis rate at 8 hours was probably little more than 10% of that at 0 hours. A similar metabolic change related to pH has been noted by Kain and Fogg (1958) who noted in culture experiments that the growth

constant for *Asterionella japonica* (as measured by change in number of cells per day) reached a maximum of 0·73 at pH 8·25, and decreased for higher values, being only 0·47 or 64% of the maximum at pH 8·5. While strict proportionality does not necessarily hold between photosynthesis and growth, it seems possible that the inhibition of metabolism associated with rising pH has been rather greater in the present experiment than in that of Kain and Fogg.

TABLE 4—Light and Dark Bottles

Date	Mean Temp. of Bath	O_2, ml/l			Gross Production mgC/m³/hr
		Light	Dark	Difference	
Two Hours					
11	12.2	6.51	6.29	0.22	59
12	12.2	6.05	5.83	0.22 ± 0.15	59
13	9.8	8.04	6.58	1.46 ± 0.05	392*
14	9.4	6.43	6.18	0.25	67
15	11.4	6.63	6.33	0.40 ± 0.08	107*
16	12.3	6.74	6.54	0.20 ± 0.10	54
17	11.7	8.17	6.95	1.22 ± 0.07	327*
17†	14.1	6.72	6.31	0.41 ± 0.04	110*
18	14.0	6.68	6.60	0.08 ± 0.04	21
19	8.3	6.53	6.46	0.07 ± 0.08	19*
20	8.4	6.61	6.45	0.16 ± 0.03	43
21	11.7	6.38	6.04	0.34 ± 0.01	91*
Four Hours					
11	12.7	7.19	6.36	0.83	111
12	13.0	6.01	5.87	0.14 ± 0.12	19
13	10.4	8.57	6.48	2.09 ± 0.13	280*
14	10.3	6.70	5.94	0.76 ± 0.18	102*
15	12.2	6.74	6.39	0.35 ± 0.08	47*
16	12.9	6.77	6.26	0.51 ± 0.02	68
17	12.3	8.81	6.84	1.97 ± 0.11	264*
.....	.....	.....	.....	.....	.....
18	14.4	6.60	6.47	0.13 ± 0.05	17
19	9.1	6.58	6.21	0.37 ± 0.05	50
20	9.1	6.55	6.29	0.26 ± 0.06	35
21	12.4	6.58	6.02	0.56 ± 0.03	75
Eight Hours					
11	.....	.....	.....	.....	.....
12	.....	.....	.....	.....	.....
13	11.1	8.84	6.43	2.41 ± 0.20	162
14	10.8	6.74	6.20	0.54 ± 0.16	36
15	13.3	7.34	5.93	1.41	95
16	13.6	7.34	6.00	1.34 ± 0.04	90
17	13.0	9.22	6.57	2.65 ± 0.15	178
17†	13.9‡	6.93‡	5.28‡	1.65 ± 0.18‡	74
18	15.0	6.37	5.96	0.41 ± 0.13	27
19	10.4	6.65	6.14	0.51 ± 0.03	36
20	10.4	6.51	6.06	0.45 ± 0.07	30
21	.....	.....	.....	.....	.....

*Significantly different from the next estimate for the same date.
†Sample at 2130 hr.
‡6 hours incubation.

A further comparison may be made between the diminution of photosynthesis and the expected decline in available CO_2. If it can be assumed that the alkalinity of the Waitarere water did not depart too greatly from that of normal ocean water, the rise in pH of 0·2 would be accompanied by a fall in partial pressure of CO_2 to about 60–70% of its initial value. This again is too small a change to explain the diminution of photosynthesis on the basis of a simple linear relationship.

A phenomenon relevant to the decline in production is the diurnal variation in photosynthesis which has been recently described (Doty and Oguri, 1957; Yentsch and Ryther, 1957; Shimada, 1958). Photosynthesis in situ reaches a maximum in the morning about 0800 hr, thereafter declining toward evening to half the peak value or less. While the present change has occurred under constant artificial illumination rather than under fluctuating natural daylight, it would seem likely that it is at least in part a manifestation of diurnal variation. It is of course also possible that diurnal variation and the above-mentioned modification of the environment are merely different aspects of the same phenomenon.

In deciding which of the three incubation times is the most valid estimate of gross production, consideration must be given to the types of change which may occur in the artificial environment of the enclosing vessel. One such change, due to photosynthesis, has been described above. A second is suggested by Steeman Nielsen (1955) who claims that different bacterial populations will develop under dark and light conditions and that respiration due to bacteria will thus be different. While this effect has been discounted by Vaccaro and Ryther (1954) for incubation periods up to 24 hours, there is little doubt that the shorter the incubation time the less the chance of deviations from natural conditions invalidating the results, and for this reason the 2 hour incubations are taken as being the most representative. While a few of these estimates are small compared with their error, it will be noted from table 4 that all are positive in sign and are in general confirmed by the longer incubation periods, but for the exceptions noted.

While the term "gross production" has been used, a more appropriate term would be "gross production at 300 foot candles", or "gross production potential", since the estimate takes no account of the actual sunlight reaching the surf during the day, but uses an arbitrary and constant illumination of 300 foot candles. (Illumination by sunlight at the sea surface may be in the order of 10,000 f.c. at noon in summer). This elimination of one of the variable factors is desirable at the present stage of the investigation, since it permits a more efficient analysis of the relationship of photosynthesis to chlorophyll *a* and to the number of diatom cells. In any case it is doubtful whether a bottle moored in the surf, as is the usual in situ experiment, would adequately reproduce the conditions in the surf itself, since constant turbulence, rapid gas exchange with the atmosphere, and many other features would be absent.

Comparison with Production in Other Areas

Even allowing for the departure from natural conditions in the incubation technique, it is evident that the rate of primary production in these waters

at the time of blooming is particularly high, reaching a maximum of nearly 400 milligrams of carbon per cubic metre per hour. By comparison, surface water in the open ocean would be considered highly productive if the corresponding figure exceeded 1 mgC/m³/hr. It has been shown by Doty and Oguri (1956) that production may increase by up to two orders of magnitude as an island mass is approached from the ocean. Unfortunately the literature does not yield much data for inshore waters, which are directly comparable with that of the present investigation. In a pre-publication data report of the University of Hawaii for 1957–58, kindly supplied by Prof. Maxwell S. Doty, the highest production rate recorded (by the ^{14}C technique, incubation at 1500 f.c.) for Pacific waters is 32 mgC/m³/hr in Manila Bay. This is in the same order of magnitude as the lowest figures recorded at Waitarere, and judging by the relative levels of incubation illumination the Waitarere estimates should be multiplied by a factor of between 4 and 5* to be comparable with Doty's estimates. Unfortunately Manila is in tropical waters, where production is commonly found to be lower than in temperate regions. It would be interesting to compare the Waitarere production with that of regions in other parts of the world which have a similar climate. Judged by visual standards, the maximum bloom recorded in the present investigation can be exceeded by at least two orders of magnitude, so that the instantaneous rate of production may at times be very great indeed.

Relationship Between Production, Chlorophyll a *and Cell Numbers*

In Fig. 3 is plotted the gross production at 300 foot candles, chlorophyll *a* content and numbers of *Chaetoceros armatum* (?). (The other plant pigments have not been incorporated in the figure, since it is generally agreed that only chlorophyll *a* plays an active part in photosynthesis).

A logarithmic scale is employed so that equivalent changes in magnitude will have the same slope for all three sets of observations. There is an obvious similarity between the three curves, more particularly in the coincidence of the two peaks on 13 and 17 August. Toward the end of the period, a third production peak appears to be commencing, though this is not reflected in the chlorophyll *a* or *Chaetoceros* curves. The chlorophyll *a* estimate on 17 August is based on the "unsettled" sample, while the abundance of *Chaetoceros* has been estimated by multiplying the figure for the "settled" phytoplankton sample by 91/14, which is the ratio of "unsettled" to "settled" chlorophyll *a* (table 4).

Of the four most abundant species other than *Chaetoceros,* three appear to have a common pattern of variation. These three species, *Asterionella japonica, Cerataulina pelagica,* and *Lauderia annulata* are plotted together in Fig. 4. The curves are quite distinct from that of *Chaetoceros,* the only point of similarity being the peak on 17 August. (The same correction

*Since 300 f.c. and 1,500 f.c. are probably both in the linear portion of the photosynthesis/light intensity curve, the factor for light would be 5. However, the ^{14}C technique is believed to measure either net production (Ryther, 1954, 1956b) or an intermediate value between net and gross production (Steeman Nielsen, 1952, Steeman Nielsen and Hansen, 1959). Thus the true conversion factor would be somewhat less than 5.

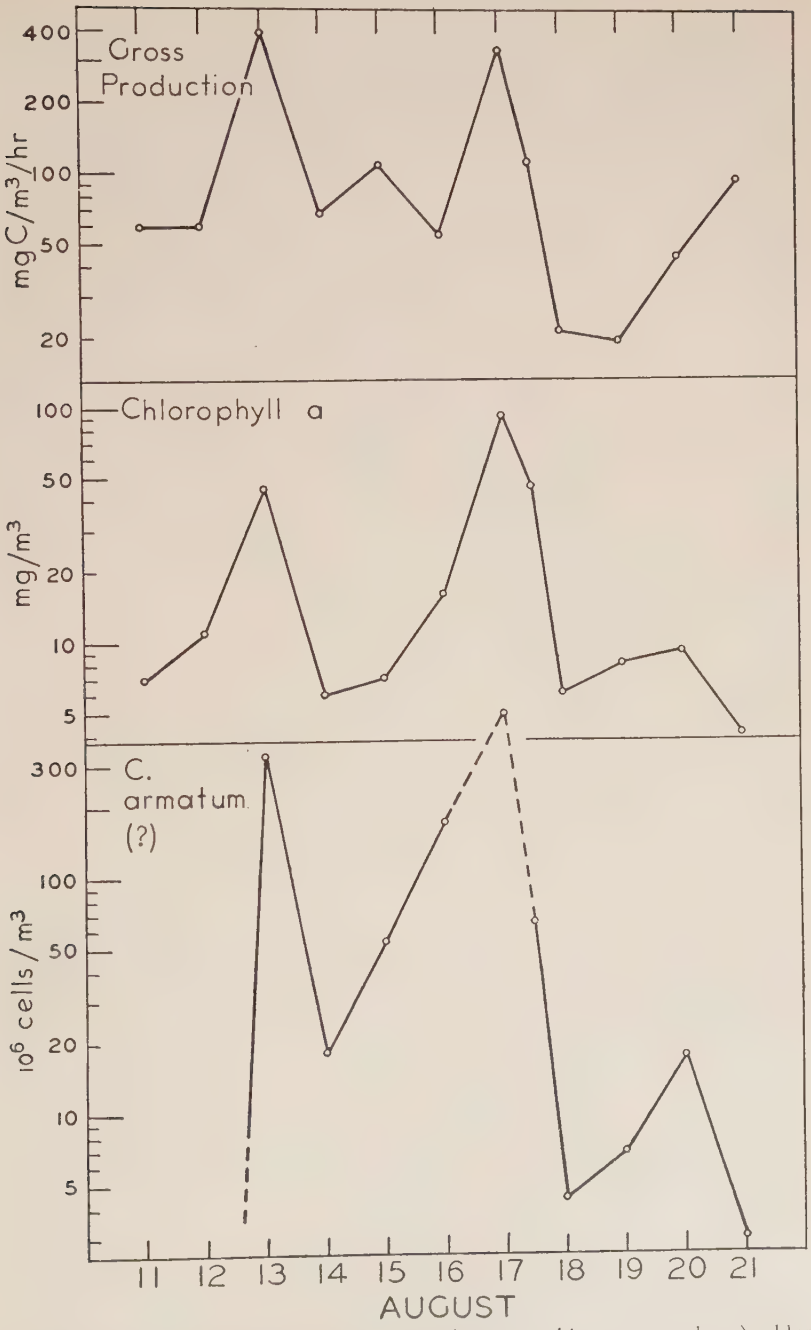

FIG. 3—Variation of gross production (mg carbon per cubic metre per hour), chlorophyll *a* (mg per m^3) and *Chaetoceros armatum* (?) (10^6 cells per m^3) on Waitarere beach, 11–21 Aug. 1959. Logarithmic scale.

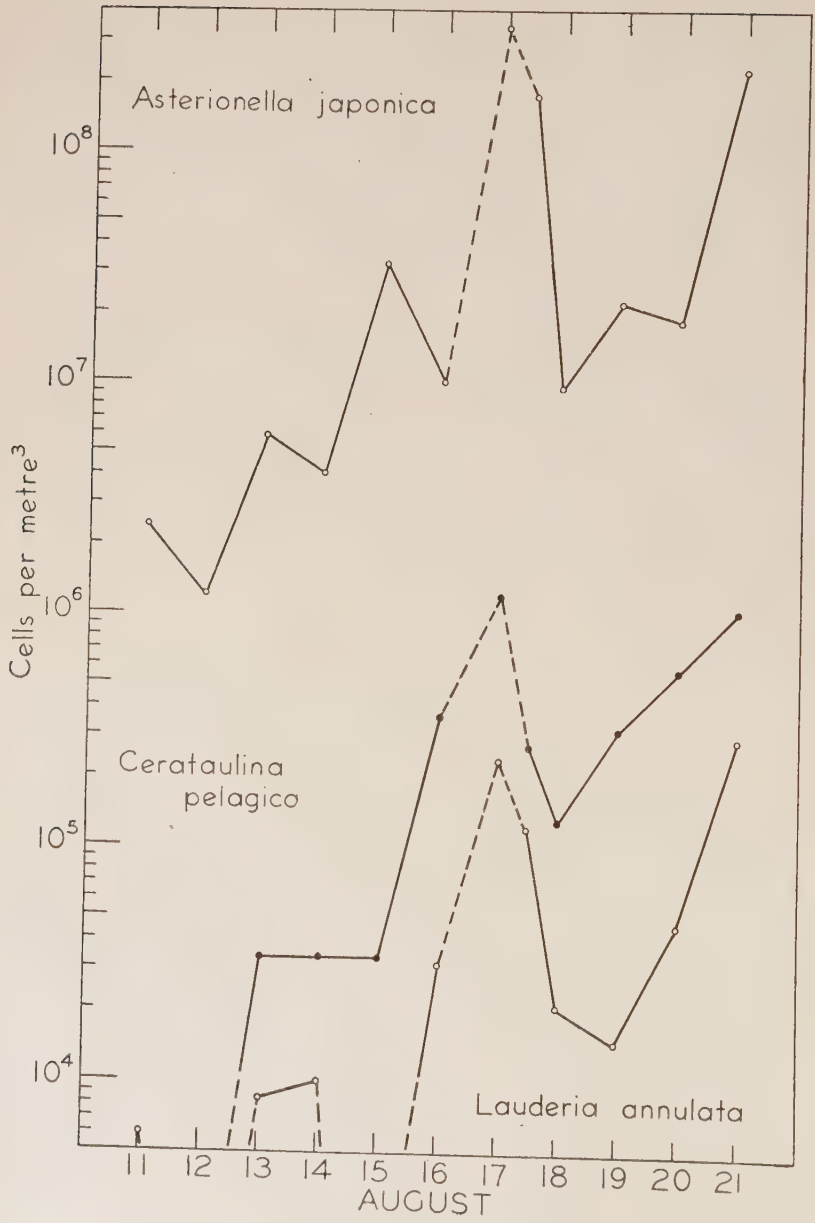

FIG. 4—Variation in abundance of *Asterionella japonica*, *Cerataulina pelagica* and *Lauderia annulata* on Waitarere beach (cells per m^3) 11–21 Aug. 1959. Logarithmic scale.

factor, 91/14, has been used for *Cerataulina* and *Lauderia* on 17 August, but for *Asterionella*, a much smaller and more buoyant species, the factor 2 is applied). However, *Grammatophora*, which is intermediate in abundance to *Cerataulina* and *Lauderia* follows a distinct pattern of its own which is apparently unrelated either to production or to other species.

In specific composition the three peaks or "blooms" in the production graph (Fig. 3) fall into three distinct categories. The first, on 13 August, is predominantly *Chaetoceros*, the second, on 17 August, *Chaetoceros* reinforced by other species, particularly *Asterionella*, *Cerataulina*, and *Lauderia*, while the third, on or after 21 August is composed almost entirely of other species, with *Chaetoceros* virtually absent.

In the first two blooms, for which the complete cycle has been observed, it is perhaps surprising that chlorophyll *a* content was not higher on 13 August, to correspond with the greater level of primary production. This, however, is almost certainly explained on the grounds that *Chaetoceros armatum* (?) is an abnormally difficult species to clear. Subsequent microscopic examination has revealed that even prolonged extraction in acetone may fail to remove all traces of chloroplast pigmentation, and it may safely be assumed that the chlorophyll content for this bloom has been underestimated. The even poorer agreement between chlorophyll *a* and production on 21 August is less easily accounted for. It will, however, be noted that this sample contained a large quantity of suspended organic matter, which may have an adverse effect on some of the determinations.

In computing correlations, both sets of estimates for 17 August are omitted, since the morning sample involves rather a crude approximation for cell numbers, while the evening sample may be biassed by diurnal fluctuation effects. Gross production has the following coefficients of correlation with:

chlorophyll *a*	0·92***
Chaetoceros armatum (?)	0·87**
Asterionella japonica	— 0·37

(** and *** indicate significance at the 0·01 and 0·001 levels of probability). If the two estimates for 17 August are included, the correlation with chlorophyll *a* falls to 0·80**. The reason for this is readily seen from Fig. 5 where production is plotted against chlorophyll *a*. The magnitude of the first correlation quoted depends almost entirely on one high-level observation for 13 August. Adding the two points for 17 August produces considerably more scatter in the diagram.

The mean regression of production on chlorophyll *a* is described by the equation:

$$P = 34{\cdot}5 + 3{\cdot}64\ Chl.\quad \text{standard error of mean} = 21{\cdot}6$$

(P = production in mg/m³/hr *Chl.* = mg chlorophyll *a*/m³).

This equation is shown as a dotted line in Fig. 5. Since the intercept, 34·5, is less than twice the standard error, it does not differ significantly from zero, so that the equation may be reduced to the form:

$$P = 4{\cdot}31\ Chl.$$

This relationship is shown as an unbroken line in Fig. 5. However, the error

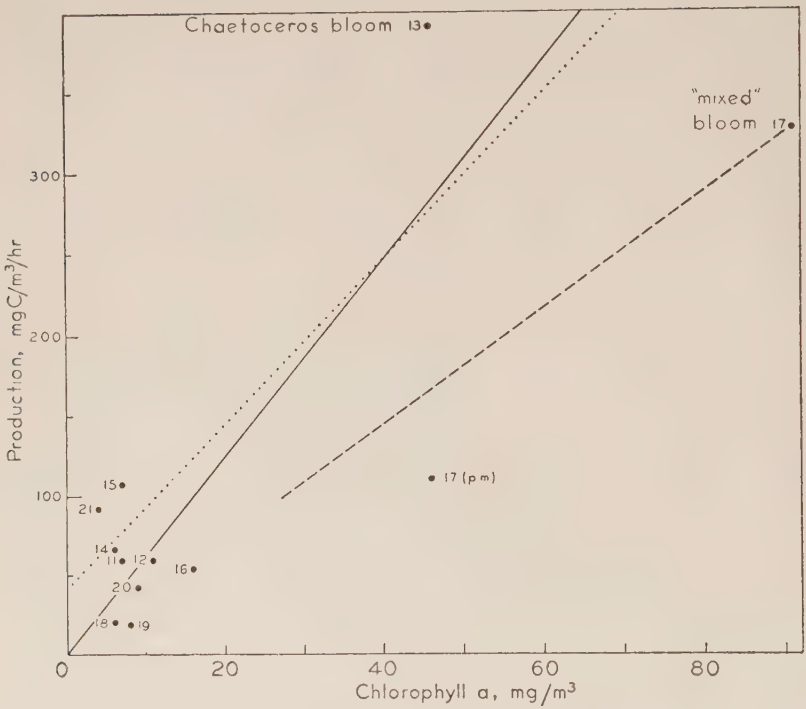

FIG. 5—Gross production (mg carbon per m^3) plotted against chlorophyll *a* (mg per m^3). Dotted line = regression. Unbroken line = regression assuming a zero intercept. Broken line = approximate regression corrected for incomplete extraction of chlorophyll *a* from *Chaetoceros armatum* (?).

introduced by incomplete extraction of *Chaetoceros* causes the regression coefficient 4·31 to be an overestimate. Probably a better estimate of the true regression is the broken line through the point for the mixed bloom (17 August), even though this line is based on one point only. The regression coefficient then becomes 3·6. Even this is probably a maximal figure since a small proportion of *C. armatum* (?) was still present on 17 August. Thus the weight of carbon fixed per hour is probably about 3–3·5 times the weight of chlorophyll *a*. This is within the same range reported by Shimada (1958) (4·24 at 2,000 foot candles), Holmes *et al.* (1957) (2·5–7·3 at 1,000 f.c.), and Ryther and Yentsch (1957) (3·7 at 2,000 f.c.). However, if allowance is made for the lower level of illumination (300 f.c.) the present figure would appear to be a relatively high one.

Owing to the specific complexity of the flora, no consistent estimate can be made of the production per cell. A crude approximation is made by ignoring all species other than *Chaetoceros,* giving the equation:

$$P = 39 + 1{\cdot}0 \times 10^{-6}\, Ch$$

(*Ch* = number of *Chaetoceros* cells)

Assuming a zero intercept:

$$P = 1{\cdot}2 \times 10^{-6}\, Ch$$

Since other species have not been taken into account, this is a maximal estimate, indicating that the average number of cells of *Chaetoceros armatum* (?) required to fix one milligram of carbon per hour is about one million.

Water Bath Temperature

It has been noted that the temperature of the water bath was not controlled and therefore varied somewhat during incubation. To ascertain whether this has had any significant effect on photosynthesis (within the limits of accuracy of the experiment) the two partial correlation coefficients were computed:

Correlation of production to chlorophyll *a* independent of temperature,

$$r_{PC.T} = 0{\cdot}83\ **$$

Correlation of production to temperature, independent of chlorophyll *a*,

$$r_{PT.C} = 0{\cdot}33$$

The chlorophyll *a* correlation is obviously not significantly higher than the total correlation ($r_{PC} = 0{\cdot}82$), while the correlation to temperature is of zero order and, if anything, of opposite sign to what might have been expected. It may be assumed, then, that bath temperature has had a negligible effect on present results.

Weather and Hydrological Conditions

The weather conditions, sea temperature, and salinity during the investigation are presented in table 5. It will be seen that there is no particular feature in common between the weather accompanying or immediately preceding the three different blooms. This is perhaps to be expected in such a short series, since the influence of weather on blooming will be indirect, probably delayed, and complicated by other factors. The principal effects to be anticipated would be firstly from wind, the direction and force of which would influence the state of upwelling of coastal waters. The greatest upwelling effect would be expected with winds from a southerly or southwesterly quarter, a direction which is fairly prevalent on this coast. Secondly, once nutrients were made available from upwelling or other sources, cloud amount would need to be such that the optimum (not necessarily the maximum) amount of sunlight was available for photosynthesis.

TABLE 5—Weather, Sea Temperature, and Salinity

Date (August)	Weather				Sea Temp. °C	Salinity ‰
	Wind	Cloud	Precipitation	Surf		
11	SW/3	3/10	.....	slight	11.1	33.2
12	ESE/3	10/10	.....	moderate	11.7	33.2
13	L. and V.	3/10	.....	moderate	10.8	33.5
14	WNW/2	9+/10	showers in afternoon	moderate	10.7	33.3
15	WNW/5	5/10	.....	rough	11.0	31.3
16	WNW/4	7/10	showers at 0830	rough	11.1	32.8
17	SSE/2	7/10	showers in morning	moderate	10.9	26.9
17(2130)	SSE/2	nil	.....	moderate	10.8	31.4
18	SSE/3	4/10	.....	slight	10.7	33.2
19	SSE/2	nil	.....	calm	9.7	32.7
20	L. and V.	nil	.....	calm	10.5	33.8
21	E/3	9+/10	.....	slight	11.6	32.9

As with weather, there is little obvious connection between sea temperature or salinity, and blooming. The temperature is relatively constant, with a range of only 2°C. Salinity is relatively low for oceanic water, suggesting some considerable influence of river and other terrigenous water. On the morning of 17 August a particularly low salinity occurred. While more rain fell this day than on any other, it is doubtful whether this could have been the direct cause. Since this sample was more than usually contaminated with suspended organic matter, it seems possible that the lowered salinity owes its origin either to the Manawatu River, about 6 miles to the north, or to the small Waiwiri stream close to the beach entrance. While, apart from this one instance, there is no obvious discontinuity in hydrological properties, both temperature and salinity are, by oceanic standards, heterogeneous, a fact which may have some bearing on the discussion in the following section.

Time Series or Space Series?

While Figs. 3 and 4 present the data as a time series, there is some possibility that the changes shown are as much a function of space as of time. Some hint of this possibility has already been given from examination of the coefficient of diversity of species, and from the heterogeneity of water properties. It was not possible to carry out a detailed sampling programme on all parts of the beach, and the blooms noted were not sufficiently intense to permit their being followed visually. However, at least two distinct concentrations of *Chaetoceros armatum* (?) were noted north of the beach entrance on occasions during the later part of the investigation, and are represented by samples 17A and 17B (table 3). These appeared to move northward fairly regularly and might well have been the same bloom noted near the beach entrance on 13 August. A rate of travel of about 5 miles in 4 days is consistent with the general northward "set" commonly found on the beach, and which was particularly noticeable during aqualung work.

CONCLUSIONS

1. A very high rate of primary production of organic matter may prevail on some North Island west coast beaches, as represented in the present instance by Waitarere Beach. The maximum figure obtained in this investigation is about 400mgC/m^3/hr at 300 foot candles, but this is probably exceeded at times by at least an order of magnitude. The dominant species in producing the associated phytoplankton blooms is usually *Chaetoceros armatum* (?) although other species such as *Asterionella japonica* may also be abundant at times. While these blooms are undoubtedly supported by a rich source of plant nutrients from upwelling water, it also seems likely that, owing to the scarcity of grazing zooplankton, a relatively rapid recycling of these nutrients takes place, so that the net production of organic material on a yearly basis might not be so great as the instantaneous gross rates of production would suggest.

1a. With such an abnormally high rate of production, it is noted that errors are introduced into the measuring technique which would normally be negligible. Estimates tend to have a low bias, owing to the fact that appreciable changes in pH and other chemical properties in the water may inhibit photosynthesis. This effect merits further investigation. It is significant, not only as a technical source of error, but also as a phenomenon which may affect the diurnal photosynthesis cycle in nature. There is a likelihood that very intense blooms, say two orders of magnitude greater than the one observed, would not be able to maintain a proportionately higher level of production. Under such circumstances, the availability of carbon dioxide, though not normally a limiting factor to photosynthesis, could be substantially reduced in a very short time.

2. The rate of gross production in relation to photosynthetic pigments is about 3–3·5 mg C per mg chlorophyll *a* per hour at 300 foot candles and a temperature of about 10°C. This is in general accordance with the results produced by other workers (or possibly somewhat higher), though results are not strictly comparable.

3. While no consistent estimate can be made of the production per cell, it is in the order of 1 mg C per 10^6 cells of *Chaetoceros armatum* (?) per hour.

4. Even though only two dominant species of phytoplankton contribute significantly to the plant pigments, it seems doubtful whether reliable quantitative estimates of species composition could be made on the basis of pigments alone. However, the two most abundant species in the present investigation may be distinguished, when one or the other is dominant, by the ratio chlorophyll *a*: chlorophyll *c*, which is less than unity for *Asterionella* and greater than unity for *Chaetoceros*.

5. The index of diversity (Fisher *et al.* 1943) for the phytoplankton population has a range between 1 and 3 (approximately), for the period of observation but is significantly heterogeneous from day to day. While the validity of this index as applied to rapidly reproducing phytoplankton is doubtful, it does appear likely, from the northward movement of visible blooms along the beach, that the changes taking place at one point are as much due to spatial as to temporal changes in the plankton population.

6. In view of the high and readily measured production rates and the frequent dominance of one or a few species, Waitarere and other similar west coast beaches may form an ideal ground in which to make future experimental studies of the phenomenon of phytoplankton blooming. Under suitable illumination intensities photosynthesis could probably be measured with significant accuracy using exposure times of an hour or less. In view of the water movements along this coast it would be necessary for determinations to be made at relatively short intervals both in space and time.

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ESTABLISHMENT OF *Procecidochares utilis* Stone (Diptera : Trypetidae) ON *Eupatorium adenophorum* Spreng. IN NEW ZEALAND

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Summary

Euaptorium adenophorum Spreng., a native of Mexico, has become established as a noxious weed in a number of countries. In New Zealand *E. adenophorum* occurs over extensive areas north of Whangarei and on the northern portion of the Coromandel Peninsula. The plant is difficult to control either culturally or with hormones.

A trypetid stem gall fly, *Procecidochares utilis* Stone was introduced to New Zealand from Australia in October 1958, and liberated on *E. adenophorum* at 2 sites on Coromandel Peninsula. *P. utilis* has established at both sites and appears to be spreading. As yet, there is no evidence that *P. utilis* is being attacked by native parasites.

INTRODUCTION

Eupatorium adenophorum, a native of Mexico, was early introduced to the horticulture trade as an ornamental flowering plant (Osborne, 1924). As a garden escape it has become a noxious weed of grazing land in a number of countries, particularly Jamaica, Hawaii, Australia, and New Zealand. The first survey of possible agents for the biological control of *E. adenophorum* was undertaken, in Mexico, by Osborn (1924) on behalf of the Hawaiian authorities. This author reported a large number of insects associated with the plant in its native habitat but no attempt was made to introduce any of these insects to Hawaii until 1945. In that year a trypetid (Tephritidae of some) stem gall fly, *Procecidochares utilis* Stone was introduced into Hawaii from Mexico. Within a few years from that time *P. utilis* had brought about effective control of *E. adenophorum* over several thousand acres (Bess and Haramoto, 1958). In 1952, *P. utilis* was introduced into Australia from Hawaii (Dodd, 1953).

This paper records the establishment of *P. utilis* on *E. adenophorum* in New Zealand.

Eupatorium SPECIES IN NEW ZEALAND

The occurrence of 2 species of *Eupatorium* in northern parts of the North Island was reported by Allan (1933). Both *E. adenophorum* and *E. riparium* C. H. Schulz, were garden escapes which had been cultivated for their abundantly produced, white, ageratum-like flowers. As neither species is well known outside the areas in which they occur, the following description adapted from Allan (1933) will serve to distinguish them. *E. adenophorum*

FIG. 1—Plants of *Eupatorium adenophorum* Spreng.

(Fig. 1) is a robust plant, with a diffuse habit; when growing in a clump it tends to be erect, up to 6 ft in height; stems are stout, dark in colour, covered with sticky glandular hairs; leaves broad, short-pointed; white flowers in dense clusters. *E. riparium* (Fig. 2), the mist flower, a low diffuse plant; stem rather woody at base, slender, reddish in colour; leaves lance shaped, taper-pointed, in opposite pairs, flower clusters on long slender stalks.

During 1954 the author surveyed the distribution and economic importance of *Eupatorium* species in the Auckland province. *E. adenophorum* appears to grow in a variety of situations although dense stands were more frequently found on bush margins. This weed also grows along stream beds; amongst scattered manuka (*Leptospermum scoparium* Forst.); on bracken (*Pteridium esculentum* Hook.) covered slopes and, occasionally, in open *Danthonia* pasture. In these situations, *E. adenophorum* is common throughout North Auckland, north of a line from Whangarei to Donnelly's Crossing as far as Kaitaia. It also occurs extensively on Coromandel Peninsula, particularly on the northern portions. In addition, infestations of

FIG. 2—Plants of *Eupatorium riparium* C. H. Schulz.

E. adenophorum have been reported from Rangitoto Island and Orere Point. The approximate distribution of *E. adenophorum* in New Zealand is shown in Fig. 3. *E. riparium* was found near Russell, in the Mangamuka Gorge, and near Te Rerenga on Coromandel Peninsula. In all cases stands of *E. riparium* occurred in shaded situations and contained small numbers of plants.

E. adenophorum appears to be a weed only on marginal land; plants were not found in areas which were adequately grazed by cattle except that, along stream banks, the weed sometimes restricts stock access to water. Opinions on the economic importance of *E. adenophorum* were varied. Near Whangarei the weed was reported to be taking charge of large areas which had been in

FIG. 3—Approximate distribution of *Eupatorium adenophorum* in New Zealand, hatched area.

bracken. In the Kaitaia district concern was expressed because of the tendency for the weed to block stream beds. A number of people held that the presence of the weed was indicative of poor stock management.

Control of *E. adenophorum* by cultural means is tedious, consisting of hand pulling of plants which are soon replaced by new seedling growth. Chemical control using sodium chlorate was discussed by Granville (1940) but this method does not always give a complete kill. More recently hormone weedkillers have been used against *E. adenophorum* without particularly successful economic results.

E. riparium in New Zealand is not considered to be a problem weed but Dodd (pers. comm.) thinks that it may be important in the future. *E. riparium* is an aggressive weed in some Pacific areas.

EFFECT OF *Procecidochares utilis* ON *Eupatorium adenophorum*

P. utilis (Fig. 4), a small marble-winged fly, oviposts on the growing point of *E. adenophorum* under the terminal pair of developing leaves. On hatching, larvae tunnel into the stem and external signs of gall formation can be seen after approximately 2 weeks (Bess and Haramoto, 1958). Towards the end of the larval period the larva tunnels to the surface of the gall leaving only a thing epidermal layer as a "window" through which the adult fly can emerge. Pupation takes place in the central chamber of the gall. Females of *P. utilis* lay an average of 175 eggs and simple galls contain a mean of

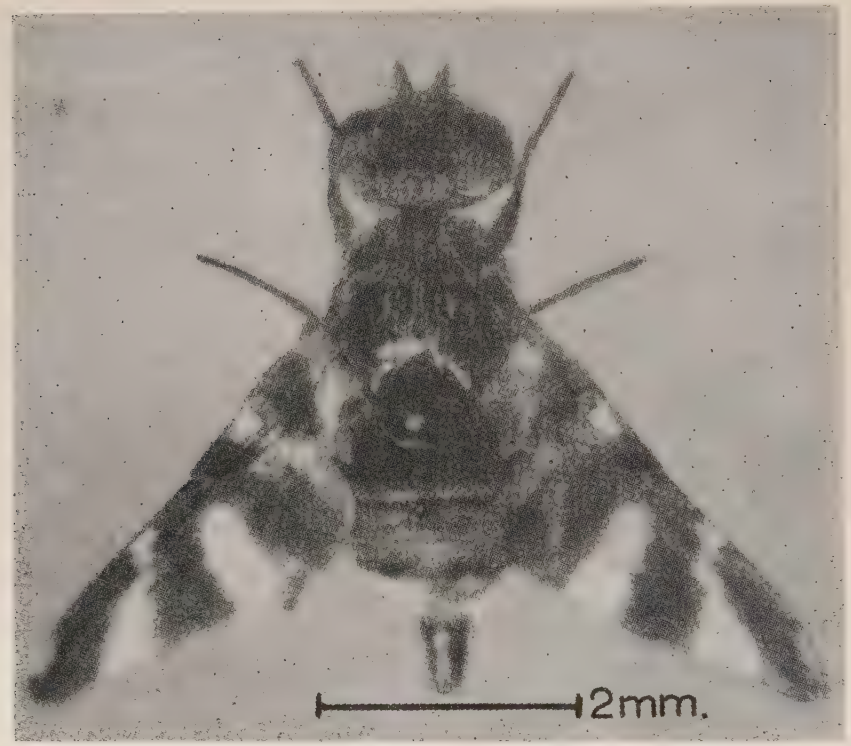

FIG. 4—Adult female of *Procecidochares utilis* Stone.

3 larvae each (Bess and Haramoto, 1958). When the fly is abundant, repeated oviposition on the growing point causes compound galls. This type of galling causes stunting of the plant and, eventually, death.

INTRODUCTION OF *Procecidochares utilis*

During 1954 the New Zealand Department of Agriculture sought information as to whether *P. utilis*, if introduced, could be expected to check *E. adenophorum* in the early stages of its establishment. Entomology Division, D.S.I.R., commenced an investigation, and in September 1956 the author had the opportunity to inspect areas of *E. adenophorum* in southern Queensland. Although *P. utilis* had been established only 4 years previously, it was widely dispersed and was causing extensive galling of its host plant in spite of a high level of parasitism of *P. utilis* by parasites of Australian species of fruit flies. Following this visit it was decided to introduce *P. utilis* into New Zealand.

A comprehensive series of food preference tests conducted by Dodd (1953) in Hawaii had shown that *P. utilis* was host specific to *E. adenophorum*. *P utilis* will oviposit on *E. riparium* but no galling or larval develop-

ment occurs on this host. Once introduction of *P. utilis* to New Zealand had been approved the major point was to ensure freedom from Australian parasites. This was met by arranging to introduce only adult flies.

TABLE 1—Consignments of *Procecidochares utilis* Adults Received in New Zealand in October 1958

	Consignment Number		
	1	2	3
Dispatched Brisbane	13th	20th	27th
Received Auckland	15th, 6 p.m.	21st, 10 p.m.	29th, 10 p.m.
Liberated	16th, 10 a.m.	22nd, 11 a.m.	30th, 8 a.m.
Number dispatched	300	188	133
Number alive at liberation	288	165	127

Through the courtesy of Mr A. P. Dodd, Director, Biological Laboratory, Queensland Department of Public Lands, 3 consignments of *P. utilis* were received in New Zealand during October 1958. Details of these consignments are shown in table 1. Of a total of 621 flies dispatched, 580 were alive at liberation, the overall mortality being 6·6%. The highest mortality (12·2%) occurred in consignment 2, which had travelled across the Tasman Sea on the flight deck of the aircraft and was not subject to delays in transit. Consignments 1 and 3 were each delayed 24 hours in transit and travelled in the unpressurised freight compartment of the aircraft. To increase the chance of establishment of *P. utilis,* consignments were collected from the aircraft at Auckland and taken to the liberation site as quickly as possible. Even allowing for delays in transit, all consignments were liberated within 72 hours of dispatch from Brisbane.

A liberation site (Fig. 5) was selected near Colville on Coromandel Peninsula; factors governing the selection of site being a plentiful supply of *E. adenophorum* and proximity to Auckland and to Entomology Division's station at Palmerston North. The chosen site was in the centre of a mixed stand of *E. adenophorum, P. esculentum,* and *L. scoparium* some 5,000 to 6,000 acres in extent, in a sheltered valley.

The original intention was to concentrate all material at the one site but due to local flooding on 16 October consignment 1 was liberated some 5 miles south of the chosen site. As this secondary site was on the fringe of the *Eupatorium* area consignments 2 and 3 were liberated at the Colville site as planned. The numbers of insects liberated at the southern and Colville sites were 288 and 292 respectively.

ESTABLISHMENT OF *Procecidochares utilis*

Since liberations were made the areas have been visited on 3 occasions. On 5 January 1959, approximately 100 galls were detected on plants within a radius of 50 yards of the Colville liberation point. At this date no evidence of galling was found at the southern site.

FIG. 5—General view of liberation site for *Procecidochares utilis*, near Colville, Coromandel Peninsula. *Eupatorium adenophorum* is distributed more or less thickly over the whole of this area.

The southern site was again visited on 5 May; around the liberation point almost all plants of *E. adenophorum* were showing conspicuous galls (Fig. 6). The patch of weed in which liberations were made is approximately 100 yards by 20–30 yards; galling appeared to have occurred uniformly over this area. In addition, galls were found on roadside plants for a distance of 500 yards on either side of the liberation point. The concentration of galls decreased with increasing distance from the liberation area. At the Colville site on 6 May, galls were found thinly dispersed on plants over a circular area of radius 200 yards from the liberation point. The overall concentration of galls and the number per plant were much lower than at the southern site.

Plants at both sites were examined for evidence of old galls low on the stems; a number of such galls were found, some showing exit holes of 4 adults. A high proportion of fresh galls in May showed windowing indicating that the occupants had either pupated or were about to do so. This was confirmed by the emergence of an adult on 7 May. A number of secondary galls were observed where the main gall had caused unnatural branching of the stems, but no windowing had occurred in these secondary galls.

FIG. 6—Stem galls on *Eupatorium adenophorum* caused by *Procecidochares utilis*, approximately natural size.

In the period October to May there had been 2 generations of *P. utilis* as shown by the spacing of galls on stems. Dodd (1953) stated that, in Hawaii, the life cycle of *P. utilis* ranged from 42–75 days, but a longer life cycle can be anticipated under New Zealand conditions.

A third visit was made to both areas on 18 July. Fresh galling had occurred at both sites; at the Colville site some galls were detected at a distance of 300 yards from the liberation point. No windowing was observed in galls at this date.

DISCUSSION

Failure to detect galling at the southern site on 5 January, 1959 may have been due to the different growth habit of plants in this area compared with those at the Colville site. The Colville area, which was inspected first, is in a drier situation; plant growth during the summer period is restricted and first generation galls were conspicuous above the bulk of the foliage. At the southern site plants were growing in a moist situation and at 5 January were almost twice as high as those in the Colville area. First generation galling at the southern site must have been lower on stems than was anticipated, the galls being hidden by foliage. This point was confirmed by finding older galls at this site during the May visit.

Although *P. utilis* was introduced to New Zealand free of parasites it is possible that it may be attacked by native hymenopterous parasites, if they exist, of other species of Trypetidae. This has been the case both in Hawaii and Australia. As yet no sign of such parasitism of *P. utilis* has been detected in New Zealand.

Differences in the apparent rate of establishment at the two sites, from similar initial liberations, are interesting. The Colville site is in the middle of a large area of *E. adenophorum* and the southern site is in a confined stand of the weed. With an insect such as *P. utilis* which is a strong flier, it may be better to make liberations in confined areas of the weed to restrict the initial dispersion of the insect, and improve its chances for mating.

Now that *P. utilis* has established in Coromandel Peninsula a period of time must be allowed for population increase before further liberations can be made in other areas of *E. adenophorum*. The ease with which *P. utilis* establishes and disperses would indicate that a relatively small number of liberations will be needed to give coverage of the main areas of the weed in Northland.

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SULPHUR ISOTOPIC VARIATIONS IN NATURE

PART 7—SULPHUR ISOTOPIC MEASUREMENTS ON SULPHUR AND SULPHATES IN NEW ZEALAND GEOTHERMAL AND VOLCANIC AREAS

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Summary

Sulphur isotopic measurements have been made on a series of sulphur samples from New Zealand geothermal and volcanic areas. The average $^{32}S/^{34}S$ ratio for fourteen "geothermal" sulphur specimens is 22.13. This is significantly lower than the average for twenty "volcanic" specimens from White Island whose average $^{32}S/^{34}S$ ratio is 22.36. A smaller number of "geothermal" and "volcanic" sulphur samples from New Guinea show the same trend. Twelve specimens have been examined in which sulphate-sulphur and sulphur were in intimate association. Wide variations in sulphur isotopic ratio were observed. An attempt has been made to correlate these variations with the biogeochemical history of the specimens.

Introduction

In previous papers in this series on sulphur isotopic variations in nature (Rafter *et al.* 1958 a and b), studies have been made on both the quantitative amount and the isotopic ratio of various forms of sulphur in samples collected from geothermal bores or volcanic fumarole discharges. The collection of such samples requires special techniques more familiar to the chemist than to the geologist who is frequently in the position to collect sulphur or gypsum samples from volcanic and geothermal areas. In this paper results are given for a series of sulphur, gypsum, and sulphate water samples from such areas.

In New Zealand, along a S.S.W. - N.N.E. active volcanic zone known as the Taupo-Rotorua graben, there are, at the southern extremity, the volcanoes Ruapehu, Tongariro, and Ngauruhoe, and at the northern, off the coast, the volcanic crater, White Island. Between these volcanic regions are numerous geothermal areas characterised by boiling pools and fumaroles. Around all these areas deposits of sulphur are abundant.

In the geothermal areas, the sulphur presumably is formed by the oxidation of H_2S. In the volcanic areas sulphur can be seen distilling from the mouths of the fumaroles. On White Island deposits of gypsum are evident

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in many parts. Samples of sulphur and gypsum from White Island were collected by one of us (I.A.K.) and by geologists of the N.Z. Geological Survey during visits in January 1955 and February 1956.

For comparative purposes, results of measurements on biogenic sulphur and sulphate (Kona and Cyrenaica), and a number of samples from the New Guinea area, have been included in this paper.

In many cases gypsum and sulphur are found in close association. A study of sulphur isotopic variations in such forms of sulphur may assist in a better understanding of the geochemical or biogeochemical processes involved in their formation. A number of such specimens have been examined and reported. The results lead to a clearer understanding of the part biological processes can play in isotopic variations in geological deposits, as is discussed in Part 8 of this series (Kaplan *et al.* 1960).

METHODS

Chemical Procedures

Before isotopic examination, sulphur specimens were purified by solution in benzene, filtering the benzene extract through a No. 41 Whatman filter paper into a platinum basin, and evaporating off the benzene on a water-bath.

Sulphur in association with gypsum was first extracted as above and the gypsum dissolved from the residue by digestion in 1 : 3 HCl, filtering to remove the sulphate-sulphur and precipitating sulphate as $BaSO_4$ by the usual analytical method.

Pure gypsum specimens were dissolved in water or dilute HCl, filtered to remove any extraneous matter and sulphate recovered as $BaSO_4$.

Sulphate waters, in the absence of silica, were treated directly with $BaCl_2$ to precipitate $BaSO_4$. After filtration the filtrate was tested with Br_2 to oxidise and recover any polythionate-sulphur that may be present. In the presence of silica, the sample was first evaporated to dryness to dehydrate silica. The salts were then dissolved in a dilute HCl solution, filtered, and sulphate recovered as $BaSO_4$.

After preparing pure sulphur or $BaSO_4$ from the original specimens, the sulphur in these specimens is converted into SO_2 for mass spectrometer examination as described by Rafter (1957).

Mass Spectrometer Measurements

All mass spectrometer measurements reported in this paper have been made in accordance with the procedures outlined by Hulston and Shilton (1958). The sulphur isotopic ratios have been measured with respect to the N.Z. Merck Sulphur Standard. A comparison of results with those of overseas workers, and with the Titahi Bay sea-water standard used originally in this laboratory, can be made by using the values for the $\delta\ ^{34}S$ or the $^{32}S/^{34}S$ ratios listed in table 1 below.

It should be noted that previous results reported from this laboratory were based on Titahi Bay sulphate as $^{32}S/^{34}S = 21{\cdot}77$. These previous results will thus have a slightly lower $^{32}S/^{34}S$ ratio than those reported here.

The term $\delta\,^{34}S$ is the difference between the $^{34}S/^{32}S$ ratio of the sample and of the standard expressed in parts per thousand (‰) of the $^{34}S/^{32}S$ ratio of the standard.

$$\delta\,^{34}S = \frac{^{34}S/^{32}S\ (\text{sample}) - {}^{34}S/^{32}S\ (\text{standard})}{^{34}S/^{32}S\ (\text{standard})} \times 1{,}000$$

$$\frac{^{32}S/^{34}S}{(\text{Sample})} = \frac{^{32}S/^{34}S\ (\text{Merck Sulphur Standard})}{1 + \dfrac{\delta\,^{34}S\ (\text{Sample})}{1{,}000}}$$

TABLE 1—Isotopic Comparison of Various Sulphur Standards

	$\delta\,^{34}S$ w.r.t. Merck Sulphur (‰)	$^{32}S/^{34}S$ and estimated error
Merck Sulphur Standard	0·0	22·10 ± 0·01
Pentlandite (Thode)	− 4·4	22·203
N.B.S. (U.S.A.) Ref. Sulphur	− 6·0	22·23 ± 0·02
Titahi Bay Sulphate (N.Z.)	+ 13·0	22·82 ± 0·02

RESULTS

In table 2 the results are given for fourteen sulphur samples collected from around some of the geothermal areas of New Zealand. The samples were from 60%–90% pure sulphur.

In table 3 results are given by the sulphur isotopic ratio for a series of sulphur samples from the volcanic crater, White Island, and for a small number of other sulphur samples from four other New Zealand volcanoes.

In table 4 the sulphur isotopic ratio for a number of samples of sulphur from volcanic and thermal regions in New Guinea are given to enable a comparison to be made between two Pacific volcanic areas, New Zealand and New Guinea.

In a previous paper (Rafter *et al.* (1958b) it was concluded that no significance, in relation to their origin, could be attached to sulphate isotopic ratios in fumarole discharges from White Island on account of the wide range of sulphur isotopic values for such sulphates. These variations could be traced to chemical and biochemical processes taking place at the fumarole vent or after the collection of representative sample of fumarole discharge.

TABLE 2—Sulphur Isotope Enrichment in Sulphur from New Zealand Geothermal Regions

Sample No.	Description and Locality	$\delta\,^{34}S$ (‰)	$^{32}S/^{34}S$
AB 364–1	Mud from banks of hot bubbling pool (T = 20°C, pH 1·55, Eh + 600mV) near Lake Rotokaua. Mud contained sulphur and sulphate	−1.6	22.14*
AB 367–1	Sulphur from Lake Rotokaua	−4.1	22.19
–2	Sulphur from Waiotapu	−1.2	22.13
–5	Sulphur Point, Government Gardens, Rotorua	−0.8	22.12
–6	Sulphur from the shore of Lake Rotorua, Government Gardens	−0.9	22.12
–7	Sulphur from Whakarewarewa, Northern Boundary opposite Arikikapakapa Golf Pavilion	+2.5	22.05
–8	"	+2.1	22.06
–9	Sulphur on rocks near Spring No. 7, Waiotapu	−0.6	22.11
–10	Deposit around small fumarole about 100 yd from Spring No. 7 away from stream	+1.2	22.08
–11	Waiotapu, near crater No. 9, between sulphur-coloured and blue lakes	−3.6	22.18
–12	Waiotapu, deposit in flow from hot spring No. 10	−3.3	22.17
R86–8	Sulphur from warm pool (T = 47°C, pH 2·15, Eh + 120 mV) in which steam and H_2S were bubbling near Lake Rotokaua	−2.0	22.15*
9A	Pool with H_2S bubbling and depositing sulphur (T = 28°C, pH 5·50, Eh − 130 mV) of west side of Lake Rotorua	−1.6	22.13*
10A	Water and sulphur from sulphur terrace (T = 68°C, pH 3·90, Eh + 370 mV) 650 ft N.E. of Venus Bath, Waiotapu	−2.5	22.16*
	Average	−1.2	22.13

*In these specimens very strong bacterial oxidation was detected.

TABLE 3—Sulphur Isotopic Enrichment in Sulphur from New Zealand Volcanic Areas

Sample No.	Description and Locality	$\delta\,^{34}S$ (‰)	$^{32}S/^{34}S$
	WHITE ISLAND		
R84–1	Schubert's Erl King fumarole – massive sulphur	—11.0	22.35
2	Seven Sleepers fumarole – sulphur around vent	—14.7	22.43
3	From steam vent at western foot of Crater ridge 10 ch. S.W. Schubert's Erl King	—10.0	22.32
4	Crest of Crater Ridge south of sulphur crater. Surface covered with fairly fresh volcanic ash coated with about ½ in. thick of coloured salts. A massive layer of sulphur 1–2 in. thick, 3–6 in. beneath the surface	—12.3	22.38
5	Seven Dwarfs fumarole – sulphur around vent	—11.1	22.35
6	Sulphur from fumarole at foot of N. wall of crater, 5 ch. west of old lake	—13.2	22.40
7	Specimen from a massive sulphur vein impregnating a large andesite boulder 4 ch. E. of Big Donald fumarole	—11.9	22.37
8	Massive sulphur encrustation on the surface of andesitic rock R84–7	—11.6	22.36
9	Sulphur from a mound on left bank of stream, 15 ch. N.W. of old factory	—14.0	22.41
10	Sulphur mound ½ ch. downstream from R84–9	—12.6	22.39
11	Sulphur mound 11 ch. N.W. of old factory	—13.0	22.39
12	Sulphur bearing sands, from one of number of large blocks of sandstone 7 ch. S.E. of Big Donald fumarole	—11.3	22.35
13	Sulphur from deposit in gully on N. side of White Island	—13.8	22.41
R364–3	Sulphur associated with sulphate from a stream N.W. of old factory	—9.7	22.32
6	Molten sulphur – Seven Dwarfs fumarole	—10.0	22.32
R86–4	Sulphur crystallised from a Seven Dwarfs fumarole and fallen as a lump on to ash at crater floor	—11.1	22.35
6A	Sulphur mixed with gypsum near beach	—14	22.4
AB364–10	Sulphur from fumarole S.E. of Seven Dwarfs	—11.6	22.36
7	Sulphur from Big Donald fumarole	—8	22.27
8	Sulphur mixed with gypsum lying in ash near old factory	—8.1	22.28
	Average	—11.7	22.36
	OTHER VOLCANIC AREAS		
R86–2A	Sulphur from Mayor Island	—8.4	22.29
11	Sulphur from Mt. Ruapehu	—13.8	22.41
AB367–13	Sulphur from inner crater, Mt. Ngauruhoe	—7.4	22.27
AB365–1	Sulphur from N.E. end of Red Crater, Tongariro	—4.1	22.19

TABLE 4—Sulphur Isotopic Enrichment in Sulphur for New Guinea Volcanic and Thermal Regions

Sample No.	Description and Locality	$\delta\,^{34}S$ (‰)	$^{32}S/^{34}S$
	VOLCANIC AREAS		
R187–1	Mt. Lingula reference LLA4	—10.2	22.33
2	„ LLA7	—10.4	22.33
	Sulphur in Association with Gypsum		
R202–1	Matupi volcano sulphur crystals from a deposit at the mouth of a vent. Temp. 99°C	—17.0	22.48
3	Rabalanakaia volcano. Four samples "A" to "D" taken from a fumarole at the base of the E. wall Temp. 99°C Sample "C"	—10.8	22.34
4	„ Sample "D"	—9.2	22.31
5	„ Sample "A"	—9.3	22.31
6	„ Sample "B"	—9.9	22.32
	Average	—11.0	22.34
	THERMAL AREAS		
R331–1	Anadu'uduu Thermal Area, Fergusson Island	—3.9	22.19
2	Mararau'sieva Thermal Area, Fergusson Island	—2.6	22.16
3	Iamelele Thermal Area, Fergusson Island	—5.6	22.23
4	Oliuna Crater – Dolin Island	—6.8	22.25
	Average	—4.7	22.21

In addition to sulphur specimens from geothermal and volcanic areas, specimens were obtained of pure gypsum crystals and of sulphur in intimate association with gypsum. A study of the isotopic variations in these specimens was made. The results for these, together with some measurements on two samples of sedimentary-biogenic sulphate and sulphur, are given in table 5, for comparison.

TABLE 5—Isotopic Ratio for Sulphur and Sulphate in Association

Sample No.	Description and Locality	*Sulphate* $\delta\ ^{34}S$ (‰)	$^{32}S/^{34}S$	*Sulphur* $\delta\ ^{34}S$ (‰)	$^{32}S/^{34}S$
AB364–1	Mud from banks of hot bubbling pool near Lake Rotokaua (Temp. 20°C pH 5·5, Eh + 600 mV)	−1.8	22.14	−1.8	22.14
R86–9	Small pool with H_2S bubbling and depositing sulphur, west side of Lake Rotorua (Temp. 28°C, pH 5·5 Eh — 130mV)	−5.4	22.22	−1.6	22.13
R86–10	Water and sulphur from sulphur terrace 650 ft N.E. of Venus Bath, Waiotapu (Temp. 68°C, pH 3·9, Eh + 370 mV)	−1.5	22.13	−2.5	22.16
AB364–3	Gypsum and sulphur from stream N.W. of old factory – White Island (Temp. 95°C, pH 1.7, Eh + 700 mV)	+8.7	21.91	−9.7	22.32
AB364–8	Sulphur mixed with gypsum lying in ash near old factory, White Island	−19.1	22.53	−8.1	22.28
R86–2	Well developed large gypsum crystals (85%) intimately mixed with sulphur (5·8%) Mayor Island	+6.6	21.96	−8.4	22.29
R86–6	Gypsum crystals intermixed with a small amount of sulphur on beach terrace – White Island	−12.0	33.37	−14	22.4
R102–8	Mud from alongside boiling mud pools contained 25% sulphur and a little sulphate $S/SO_4^= = 10/1$. 10 ch. S.S.E. Big Donald Fumarole, White Island	−7.0	22.26	−14.7	22.43
R92–14	Cyrenaicea sulphur, Cyrenaican Lakes, North Africa	−2.4	22.16	−20.2	22.56
R152–6	Kona sulphur (Masulipatam) east coast of India	+19.6	21.68	+19.0	21.69
R187–2	Gypsum associated with sulphur, Mt. Lingula, New Guinea	+4.4	22.01	−10.4	22.33
R202–2	Gypsum crystals dispersed in matrix of rock fragments and clay from inner eastern wall of Matupi volcano	−11.0	22.35		
R202–1	Sulphur crystals from mouth of vent (Temp. = 99°C, H_2S present) Matupi volcano			−17.0	22.48

DISCUSSION

In Fig. 1 has been tabulated the range of $^{32}S/^{34}S$ ratios for results shown in tables 2 to 5. For comparative purposes, the New Zealand Merck sulphur standard, New Zealand sea-water standard, Thode's Pentlandite secondary standard, and N.B.S. standard sulphur are clearly marked. Results from previous papers in this series, Rafter *et al.* (1958 a and b) have been indicated on Fig. 1 to show the average value for sulphur isotopic ratio for geothermal bore discharge sulphur and for fumarole discharges from the volcanic crater, White Island.

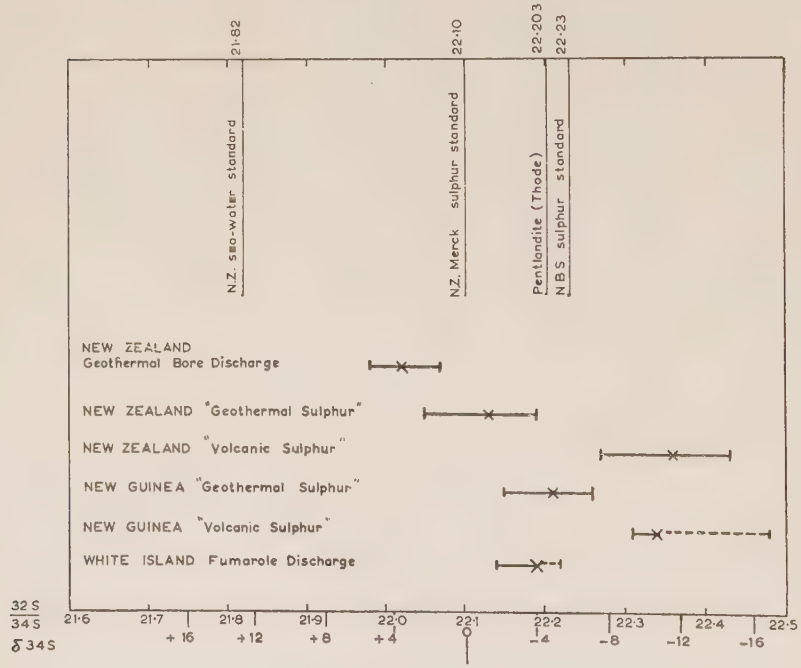

FIG. 1

The evidence presented in this paper shows that sulphur associated with the cooler geothermal regions in both New Zealand and New Guinea is not as enriched in the lighter sulphur isotope as is volcanic sulphur from these regions. In geothermal areas, sulphur is presumably formed by the oxidation of H_2S according to the equation

$$H_2S + \tfrac{1}{2}O_2 \rightleftarrows H_2O + S^\circ$$

Such a reaction should yield no fractionation under isotopic equilibrium conditions, according to the calculations of Tudge and Thode (1950). A laboratory experiment with a Kipps gas generator in which there was atmospheric oxidation of the H_2S to sulphur above the acid, gave an enrichment of

3 ‰ for ^{34}S in the sulphur formed. An enrichment of this same order is shown for New Zealand geothermal sulphur with respect to the sulphide in geothermal bore waters.

It has been shown previously by Rafter *et al.* (1958b), that the $\delta\,^{34}S$ values for sulphur around the vents of fumaroles and that precipitating out of fumarole discharge, both during and after collection, are isotopically the same within the limits of our mass spectrometer measurements. It was concluded that this so-called "volcanic" sulphur was enriched in the ^{32}S isotope by chemical reactions at the vent mouth, with respect to sulphur in the parent magma. From results in this paper for "volcanic" sulphur from both New Zealand and New Guinea, it is considered that this sulphur has been enriched by similar chemical reactions. These chemical reactions at White Island appear to involve the formation of polythionates. The sulphur separating from such a solution is isotopically enriched in ^{32}S with respect to the more stable polythionates. The sulphate in solution is still further depleted in the lighter isotope.

In table 5, a series of isotopic values for sulphate and sulphur in association with each other has been tabulated. In four cases the sulphur and sulphate are isotopically the same, while in other cases the sulphate can be isotopically enriched in ^{34}S with respect to the sulphur by as much as 18 ‰ or isotopically depleted by 10 ‰.

It is evident that very careful attention must be paid to the biogeochemistry of such specimens before any significance can be given to the results.

Kaplan (1956), in a survey of the New Zealand geothermal regions, found ample evidence of bacterial oxidation of reduced sulphur compounds, and of sulphate reduction in more restricted areas. He was able to get bacterial growth of sulphur oxidisers which produced pH 1·0, while Parker (1951) noted that thiobacilli often cause formation of sufficient sulphuric acid in sewers to drop the pH from 7 or 8 to lower than 1·0. Butlin (1953) quotes cases where sulphur oxidising bacteria were able to function in 10% H_2SO_4 at pH 0·6. This demonstrates that bacterial activity is at least possible in most geothermal regions. This has generally been disregarded as can be witnessed by the recent article by Sakai (1957) who assumes no biological activity in "Yugama" crater lake of Mt. Shirame because the pH is 0·8.

Although it is often difficult, if not impossible, to differentiate between bacterial and atmospheric oxidation of sulphur compounds, the finding of active bacteria and pools of relatively high acidity, in a region where the ground water is neutral, often indicates an active role played by bacteria. The hot spring areas of Rotokaua, Rotorua, and Waiotapu described in table 5, fit into this category. The possible effects of bacterial activity can be seen in the crater of volcanoes such as Matupi (R.202/1–2, table 5) and on White Island. Two samples (AB364/8 and R86/6) in table 5 display this particularly well. In each case they were found partly buried in ash or clay in an area where little or no activity was present. The sulphur associated with the gypsum appears to have been derived from volcanic or magmatic activity. The sulphate was intimately associated with the sulphur, and probably derived from it after burial. In sample AB364/8, for example, the sulphur only was exposed to the surface, while the part embedded in the ash and clay contained the gypsum.

The necessity for a careful environmental study of the history of sulphur specimens after deposition is shown by the reversal of the isotope enrichment in the sulphur for specimens from the Cyrenaican Lakes, North Africa, and Kona Sulphur, India (R92/14 and R152/6, table 5). These specimens are more fully discussed by Kaplan *et al.* (1960).

CONCLUSIONS

1. Geothermal areas contain elementary sulphur, apparently formed as a result of atmospheric oxidation of H_2S. This sulphur is depleted in the ^{32}Sulphur isotope with respect to sulphur from volcanic areas by approximately 1%.

2. Wide variations can be found in the $^{32}S/^{34}S$ ratio of sulphur and sulphate in association with each other. Little significance can be attributed to such isotopic ratios unless the geochemical or biochemical history of the specimens is accurately known.

3. The sulphur isotope variations found in sulphur specimens from geothermal and volcanic areas in New Guinea follow the same pattern as found for similar areas in New Zealand.

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PHYSIOLOGICAL STUDIES OF SOME COMMON FUNGI FROM GRASSLAND SOILS

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Summary

A study has been made of some of the physiological properties of common fungi in New Zealand soils under tussock-grassland and introduced pastures. The following fungi have been used: non-sporing fungi, *viz.* Sterile Mycelia, a *Rhizoctonia* sp., and *Rhizoctonia solani,* and sporing fungi, *viz. Cylindrocarpon radicicola, Fusarium culmorum, Fusarium sambucinum* var. *coeruleum,* and *Fusarium oxysporum.*

In general, the Fusaria grew best at 28°C. All fungi grew well at 16°–24°C and in media of pH 5–8.

The Sterile Mycelia and a strain of *Rhizoctonia solani* required thiamin, or "pyrimidine", but the other fungi did not require growth factors. Some strains responded markedly to the addition of zinc.

The nutrition of the fungi was studied on different carbon and nitrogen substrates.

The sporing fungi generally utilised a greater range of carbohydrates and alcohols than the non-sporing fungi. High mycelial yields usually resulted on most of the simple carbohydrates tested. The fungi did not produce appreciable amounts of antibiotic compounds, organic acids, or non-volatile metabolic products soluble in organic solvents. Glucose carbon was assimilated very efficiently, particularly by the non-sporing fungi. The mycelia of *Cylindrocarpon* and the Fusaria contained much fatty material but little was present in the non-sporing fungi. Mannitol was isolated from mycelia as a metabolic product.

Nearly all strains utilised pectin efficiently and could utilise hemicellulose and cellulose.

Acids of the Krebs' citric acid cycle and simple aromatic compounds from lignin were, in general, utilised efficiently by *Cylindrocarpon* and the Fusaria but poorly by the non-sporing fungi.

The fungi could utilise the nitrogen of nitrate, ammonium compounds, proteins, nucleic acids, uric acid, and, generally, of purines. *Cylindrocarpon* could utilise uracil but pyrimidines were generally not metabolised.

Cylindrocarpon and the Fusaria had similar nutritional properties and could use a greater range of substrates than the Sterile Mycelia and Rhizoctoniae which themselves showed a marked resemblance to each other. No major differences were found in the nutrition of different strains of a particular species from different grassland soils or between strains which were predominant in some soils and occurred only infrequently in others.

Although it is not possible to state which properties would be most important for growth of these fungi in soil, it can be concluded that their general growth characteristics and nutritional capabilities would be of considerable ecological importance.

General Introduction

Sterile Mycelia, a *Rhizoctonia* sp., *Rhizoctonia solani., Cylindrocarpon radicicola* and Fusaria were commonly found by Thornton (in press) in New Zealand soils under tussock grassland, introduced pastures, and broadleaf forest. Important physiological differences must exist between these species as

different species were shown to be predominant in different soils, *e.g.* the *Rhizoctonia* sp, and *Cylindrocarpon radicicola* were predominant in tussock-grassland soils and *Fusarium oxysporum* in some soils under introduced pasture. Because of the abundance of these fungi in grassland soils, it seems likely that they play a considerable part in soil processes, particularly in the decomposition of plant roots. As their physiological properties are mainly unknown, a few strains have been chosen for investigation. In this work, an attempt has been made to compare the properties of different species but, in view of strain variations and the small number of species examined, only marked characteristics have been sought. The influence of different environments on the physiological properties of strains of a particular species has been studied by comparing strains which occurred either predominantly or infrequently in different soils.

The above species of fungi have often been recorded in soils in other countries and are considered to be amongst the most abundant of saprophytic soil fungi. These fungi have also been found to be commonly associated with living and dead plant roots, *e.g.* some sterile mycelia and Rhizoctoniae have been recorded by Harley (1950) and Waid (1957), *Cylindrocarpon* by Kurbis (1937), Harley and Waid (1955), Peterson (1958) and Stenton (1958), and Fusaria by Waid (1957), Griffiths and Siddiqi (1958) and Stenton (1958). The strains of the Sterile Mycelia and Rhizoctoniae studied here and isolated from tussock-grassland soils have been found to be non-pathogenic for several common host plants (Morrison *et al.* 1959) but many Rhizoctoniae and Fusaria which live saprophytically in the soil are pathogenic for plants. Although studies on the nutrition and metabolism of Rhizoctoniae have previously been conducted with strains isolated from infected plant materials, similar studies do not appear to have been carried out with strains obtained directly from soil. Very few studies have been made of the physiology of *Cylindrocarpon*. Fusaria have been examined more often, as some strains produce metabolic products of biochemical interest and others are important as plant pathogenic fungi.

Much of this paper is devoted to a study of the nutrition of the fungi on media containing different carbon and nitrogen substrates. These results should reflect the ability of the fungi to utilise available substrates in soil. The influence of temperature and pH on growth are also studied and a brief search has been made for metabolic products and antibiotic compounds. In spite of the limited number of tests performed, it is hoped that the present study will contribute to the understanding of the ecology of fungi in soil.

For convenience, the report is divided into nine sections. Except for the general introduction and final discussion, each section comprises materials and methods, and results.

General Materials and Methods

Organisms

All fungi were isolated from screened immersion-plates (Thornton 1952) and purified on potato-glucose agar. The stock cultures of the Sterile Mycelia, Rhizoctoniae and *Cylindrocarpon radicicola* were then maintained on Czapek-

Dox agar. The Fusaria were maintained on potato-glucose agar, a medium which is commonly used to prevent their degeneration in culture. The cultures were kept at about 4°C and subcultured at three- to six-monthly intervals. These strains have been disposed in the culture collection of the Commonwealth Mycological Institute; their herbarium numbers are shown in table 1. The *Rhizoctonia* sp. and *Rhizoctonia solani* were kindly identified by Dr A. Kerr, Waite Agricultural Research Institute, University of Adelaide.

Sterile Mycelium 1T is identical with fungus S.B. 235 described by Thornton (1958). Morphologically, Sterile Mycelium 2P was the same. On Czapek-Dox agar, these strains produced small sclerotia, off-white in colour, usually 1–3 mm diameter, upon aerial, white, cottony mycelium composed of septate, even hyphae, 2–3μ diameter.

Morphologically, the three strains of the *Rhizoctonia* sp. were very similar to each other. On Czapek-Dox agar, the mycelial felts of strains 3T and 5P were dull white in colour, with off-white sclerotia. The surface mycelium of the more vigorously growing strain, 4T, was also white, but the reverse was yellow or orange. Dark brown sclerotia developed from the rapidly spreading fawn-brown mycelia of *Rhizoctonia solani* strains 6T and 7P.

On Czapek-Dox agar, all strains of *Cylindrocarpon radicicola* produced brown mycelia and the medium, after growth, was also brown-dark brown in colour. In liquid media, they generally produced more submerged growth than the other fungi studied.

On potato-glucose agar, *Fusarium culmorum* developed a fluffy mycelium with a white-pink upper surface and a deep red reverse. *Fusarium sambucinum* var. *coeruleum* grew more slowly and produced a white-gold mycelium with a red reverse. *Fusarium oxysporum* strains 14P, 15P, and 16P produced somewhat fluffy growth with a deep red-purple reverse and pigmented media. The reverse of strain 17T was variegated with red and blue patches. Strains 18P and 19P were less pigmented and the reverse growth was usually dull green-blue. On most liquid media, strain 17T produced rather mucoid mycelial felts and many microconidia in the culture fluids which acquired an alcoholic odour. The other strains of *Fusarium oxysporum* did not produce many conidia in the culture fluids.

A description of the sites from which the fungi were isolated is presented in table 1, together with an indication of the frequency of occurrence of each species in the soil from which it was obtained.

Chemicals

A description of most of the chemicals used is given by Ross (1960). K_2HPO_4 and uric acid were obtained from British Drug Houses Ltd., hemicellulose from L. Light and Co. Ltd., gelatin from Davis Gelatine (N.Z.) Ltd., and albumen (from blood), casein (soluble, light white) and gluten (from wheat flour) from Hopkin and Williams Ltd. "Thiazole" (5-(2-hydroxyethyl)-4-methyl-thiazole) and "pyrimidine" (4-amino-5-aminomethyl-2-methylpyrimidine dihydrochloride) were obtained from Roche Products Ltd.

All chemicals were used as supplied by the distributors and were not further purified.

Table 1—History of Cultures

	Fungus	Present Strain Number†	Details of Isolation: Isolation Site	Details of Isolation: Soil Type	Frequency of Occurrence (%)††	Commonwealth Mycological Institute Herbarium Number (I.M.I. No.)
Non-sporing fungi	Sterile Mycelium	1T	Waiouru	Taupo hill soil	5	71568
		2P	Marton	Kiwitea silt loam	2	71569
	Rhizoctonia sp.	3T				–
		4T	Waiouru	Taupo hill soil	41	71570
		5P	Marton	Kiwitea silt loam	4	71571
	Rhizoctonia solani Kühn	6T	Alexandra	Omarama steepland soil	2	71572‡
		7P	Marton	Kiwitea silt loam	2	71573‡
Sporing fungi	*Cylindrocarpon radicicola* Wollenw. ..	8T	Waiouru	Taupo hill soil	20	71574
		9F	Marton	Kiwitea silt loam	32	71575
		10P				71576
		11P	Marton	Kiwitea silt loam	7	71577
	Fusarium culmorum (W. G. Smith) Sacc., det. W. L. Gordon	12P	Marton	Kiwitea silt loam	16	71578
	Fusarium sambucinum Fuchel var. *coeruleum* Wollenw.	13T	Waiouru	Taupo hill soil	2	71579

Fusarium oxysporum Schlecht. ex Fr. ..	14P				71580
	15P	Claudelands	Horotiu sandy loam	39	71581
	16P	Marton	Kiwitea silt loam	25	71582
	17T	Waiouru	Taupo hill soil	3	71583
	18P	Marton	Kiwitea silt loam	25	71584
	19P	Korokoro	Korokoro silt loam	22	71585

† The vegetational covers of the soils from which the cultures were isolated are indicated by:

T = tussock grassland;

P = introduced pasture;

F = broadleaf forest.

†† Indicates frequency of occurrence of each species, expressed as a percentage of the total number of fungi isolated at that site (Thornton, in press).

‡ These cultures have been disposed in the herbarium of the Commonwealth Mycological Institute as *Corticium solani* (Prill. and Delacr.) Bowd. and Golz.

Media

All media were sterilised at 15 lb/in.^{2} for 15 min. Unless otherwise stated, all media for the growth of the Rhizoctoniae were adjusted to pH 6·5 and for the Sterile Mycelia, *Cylindrocarpon* and the Fusaria to pH 6·0 before sterilisation.

Media for General Growth and Maintenance

The following media have been used:

Czapek-Dox medium: KH_2PO_4, 1·0 g; KCl, 0·5 g; $MgSO_4 \cdot 7H_2O$, 0·5 g; $FeSO_4 \cdot 7H_2O$, 0·01 g; $NaNO_3$, 2·0 g; glucose, 50 g; distilled water, 1 l.

Raulin-Thom medium (Smith 1947): Ammonium tartrate, 2·67 g; $(NH_4)_2HPO_4$, 0·40 g; $(NH_4)_2SO_4$, 0·17 g; K_2CO_3, 0·40 g; $MgCO_3$, 0·27 g; $FeSO_4 \cdot 7H_2O$, 0·05 g; $ZnSO_4 \cdot 7H_2O$, 0·05 g; tartaric acid, 2·67 g; glucose, 50 g; distilled water, 1 l. Glucose was added after separate sterilisation.

Richard's medium (Matsumoto 1921): NH_4NO_3, 1·0 g; KNO_3 0·5 g; KH_2PO_4, 0·25 g; $MgSO_4 \cdot 7H_2O$, 0·25 g; peptone, 20 g; glucose, 50 g; distilled water, 1 l; pH 6·0. Glucose was added after separate sterilisation.

Potato-glucose agar: Washed, chopped potatoes (200 g) were steamed for 30 min. with tap water (1 l) and strained through coarse muslin. Glucose (20 g) was added to the filtrate and the medium adjusted to pH 5·5.

Where necessary, media were solidified by the addition of agar (1·5%).

Media for Nutritional Experiments

Basal salts solution: The basal salts solution used for experiments reported in tables 6, 7, 9, 10, 11, and 12 contained KH_2PO_4, KCl, $MgSO_4 \cdot 7H_2O$ and $FeSO_4 \cdot 7H_2O$ and was identical with the Czapek-Dox medium but with $NaNO_3$ and glucose omitted.

In media containing nitrate-nitrogen, the carbon compounds were usually added before sterilisation. In media containing other forms of nitrogen, however, carbon compounds were added after separate sterilisation.

Cultural Conditions

Culture Vessels

Unless otherwise stated, the fungi were grown in 100 ml conical flasks with 20 ml medium.

Inocula

Disks (6 mm diameter), cut from the edge of growing colonies on Czapek-Dox agar (pH 6·0), were used as inocula in all experiments. One disk was used for each flask and was floated on the medium with the hyphal side uppermost.

Incubation

All liquid cultures were incubated in the dark without shaking. Although growth in static cultures usually takes place under sub-optimal concentrations of oxygen, the method was considered suitable for the present study. In the soil, it is probable that the fungi would also, at times, grow under conditions of low oxygen tension.

Temperature of Incubation

Carbohydrate utilisation was studied mainly at room temperature (about 12°–25°c). In the other experiments, unless otherwise stated, cultures of the Rhizoctoniae were incubated at 20°c and all other species at 24°c.

Periods of Incubation

Two incubation periods were selected for most of the nutritional experiments. The first period gave about the maximal yields of mycelial felts in the Czapek-Dox medium, supplemented with thiamin for the Sterile Mycelia and *Rhizoctonia solani* 6T. The longer period was chosen for those media in which substrate utilisation was slow. Yields have been recorded usually for the shorter period. However, if yields were at least 10 per cent greater after the longer period, these yields have then been recorded and marked in the appropriate tables. The incubation periods selected were as follows: Sterile Mycelia and Rhizoctoniae, 28 and 56 days; *Cylindrocarpon radicicola,* 21 and 28 days; Fusaria, 14 and 21 days.

Measurement of Growth

Growth in the same medium was sometimes estimated by measuring diameters of colonies. In different media, however, a strain often produced markedly different mycelial felts which could not be accurately compared by this method. In most experiments, therefore, growth was assessed by obtaining dry weights of mycelial felts in single or duplicate flasks. The mycelial felts were separated by filtration on a Buchner funnel and washed with cold water. The agar inoculum disks were removed and the felts separated from the filter paper and dried overnight at 105°c.

Presentation of Results

For convenience, yields of fungal material are recorded in the tables as yields of mycelium; these yields, however, include any spores present.

Strains differed considerably in their vigour of growth and in their yields of mycelial felts. In tables 6, 9, and 12, the yield of each strain from each substrate has, therefore, been expressed as percentage of its highest yield, i.e., on the optimal substrate, in each experiment. A figure of 100 signifies the optimal substrate. Also given are the yield in milligrammes with the optimal substrate and the mean yield and coefficient of variation for each strain. It is hoped that, by this means, the preferences of the strains for the substrates tested can be readily seen.

INFLUENCE OF TEMPERATURE AND pH ON GROWTH

Materials and Methods

MEDIA

The Sterile Mycelia and Rhizoctoniae were grown on Richard's medium and *Cylindrocarpon* on Czapek-Dox medium. The Fusaria were grown on potato-glucose agar in the temperature experiments and on Czapek-Dox medium in the pH experiments. Media were adjusted, before sterilisation, with 2N-HCl or 2N-NaOH. In the temperature experiments, the media were solidified with agar.

PERIODS OF INCUBATION

No significance is attached to the different incubation times which were chosen for convenience. As the media were not strongly buffered, cultures in the pH experiments were incubated for short periods only.

MEASUREMENT OF GROWTH

The diameters of the colonies reported in tables 2 and 3 were obtained by subtracting the diameter of the inoculum disk from the total diameters of the colonies measured.

Results

INFLUENCE OF TEMPERATURE ON GROWTH

In general, all strains grew well at 20°C (table 2).

The optimal temperature for growth of all strains of the Sterile Mycelium and *Rhizoctonia* sp. was about 20°C. All, except strain 3T, grew better at 24° than at 16°C. *Rhizoctonia solani* grew best about 24°C and growth was still vigorous at 28°C. Similar temperature responses were found by Matsumoto (1921) and Houston (1945) for strains of *Rhizoctonia solani* pathogenic for plants.

All strains of *Cylindrocarpon radicicola* grew slightly better at 20°C than at 24°C. At 28°C, growth had almost ceased.

In general, the Fusaria had high optimal temperatures for growth. *Fusarium culmorum* and *Fusarium sambucinum* var. *coeruleum* grew best at 24°–28°C. Ashley *et al.* (1937) also found 24°C to be optimal for *Fusarium culmorum*. Buxton (1955) found that maximal growth of *Fusarium oxysporum* occurred at about 25°C. Five of the strains of *Fusarium oxysporum* studied here grew best at about 28°C and still vigorously at 30°C or 32°C. The other strain, 17T, had an optimum nearer 20°C but it still grew well at 28°C.

Fusarium oxysporum strains 14P and 15P produced most pigment below 28°C but more microconidia and macroconidia at 30°C. The sporulation of strains 16P, 18P, and 19P was not markedly increased at the higher temperatures.

INFLUENCE OF pH ON GROWTH

These experiments were made to determine the most suitable initial pH of media for the nutritional studies. No account has been taken of any changes in the pH values of the media reported in table 3 which may have occurred as a result of sterilisation and growth.

TABLE 2—Influence of Temperature on Growth

Strain		Diameters of Colonies (mm) at Incubation Temperature (°c)								Incubation Time (Days)
		10°†	14°†	16°†	20°	24°	28°	30°	32°	
Sterile Mycelium	1T	19	–	29	*42*	41	28	–	–	14
	2P	–	–	10	18	*19*	11	–	–	7
Rhizoctonia sp.	3T	22	–	*41*	*41*	22	2	–	–	14
	4T	–	–	22	*49*	46	5	–	–	4
	5P	–	–	3	*28*	22	1	–	–	7
Rhizoctonia solani	6T	–	–	48	59	*62*	–	0	–	7
	7P	–	–	50	76	*86*	75	–	–	4
Cylindrocarpon radicicola	8T	–	–	40	*46*	45	–	2	–	7
	9F	–	21	–	*46*	44	1	–	–	7
	10P	–	12	–	*30*	27	0	–	–	7
	11P	–	20	–	*43*	41	2	–	–	7
Fusarium culmorum	12P	–	–	31	47	*60*	57	28	–	3
Fusarium sambucinum var. *coeruleum*	13T	5	–	–	31	31	*33*	–	–	3
Fusarium oxysporum	14P	–	–	21	31	38	*43*	41	–	3
	15P	–	–	14	23	27	*31*	*31*	–	3
	16P	–	–	19	28	34	*40*	–	23	3
	17T	6	–	–	27	23	14	–	–	3
	18P	–	–	9	17	24	*35*	–	16	3
	19P	–	–	18	27	36	*37*	–	31	3

– Signifies no determinations made.
† Approximate temperature, *viz.* room temperature.
Approximate maximal yields are in italics.

All strains grew satisfactorily within the pH range 5–8 (table 3). Although there was often no well-defined optimal pH, the Sterile Mycelia, *Cylindrocarpon* and the Fusaria usually grew best at about pH 6·0 and the *Rhizoctoniae* at a slightly higher pH. Matsumoto (1923) showed that the optimal pH for growth of *Rhizoctonia solani* depended considerably on the nature of the medium.

Under strongly acid conditions, growth of the non-sporing fungi was markedly depressed whereas nearly all strains of *Cylindrocarpon* and the Fusaria grew moderately well in media of pH 3·0.

The pigmentation of *Fusarium culmorum* and *Fusarium oxysporum* strains 14P and 15P was most intense below pH 6·0 and decreased in alkaline media.

TABLE 3—Influence of pH on Growth

Strain		Diameters of Colonies (mm) on Media With Initial pH									Incubation Time (Days)
		3	4	5	5.5	6	6.5	7	7.5	8	
Sterile Mycelium	1T	0	5	9	10	12	12	12	9	9	5
	2P	–	6	11	13	12	11	12	11	9	4
Rhizoctonia sp.	3T	0	1	2	3	3	3	4	3	2	7
	4T	3	8	13	16	16	16	18	16	16	4
	5P	0	3	12	12	13	15	15	12	11	7
Rhizoctonia solani	6T	0	5	13	14	18	18	18	17	14	3
	7P	–	36	60	60	64	64	66	63	58	4
Cylindrocarpon radicicola	8T	11	12	21	21	23	22	20	21	19	5
	9F	5	–	14	–	21	–	14	12	–	5
	10P	2	11	19	20	24	23	24	22	19	7
	11P	6	7	10	11	17	13	14	13	11	5
Fusarium culmorum	12P	12	16	32	35	37	36	36	36	33	3
Fusarium sambucinum var. *coeruleum*	13T	1	9	11	13	15	12	11	–	10	3
Fusarium oxysporum	14P	6	20	20	22	22	22	21	21	18	3
	15P	11	14	17	15	17	16	17	17	17	3
	17T	7	18	22	23	22	19	18	16	13	3

– Signifies no determinations made.

INFLUENCE OF GROWTH FACTORS AND TRACE ELEMENTS ON GROWTH

Materials and Methods

MEDIA

Czapek-Dox medium (50 ml) in 250 ml conical flasks was used as the basal medium.

Media for Growth Factor Studies

Growth factors (table 4) were added as follows: thiamin, 1 p.p.m.; nicotinic acid, 1 p.p.m.; riboflavin, 1 p.p.m.; calcium pantothenate, 0·5 p.p.m.; pyridoxine, 0·5 p.p.m.; biotin, 0·2 p.p.m.; p-aminobenzoic acid, 0·1 p.p.m.

Thiamin, "thiazole" and "pyrimidine" were used at a concentration of 1·0 p.p.m.

Media used in table 5 contained trace elements at the concentrations given below.

Media for Trace Element Studies

Thiamin (1·0 p.p.m.) was present in all media used for studying the influence of trace elements on growth of the non-sporing fungi (table 4). The concentrations of trace elements and, in parentheses, the salts as which they were added, were as follows:

iron ($FeCl_3 \cdot 6H_2O$), 2·6 p.p.m.; zinc ($ZnSO_4 \cdot 7H_2O$), 0·4 p.p.m.; copper ($CuSO_4 \cdot 5H_2O$), 0·1 p.p.m.; manganese ($MnCl_2 \cdot 4H_2O$), 0·05 p.p.m.; molybdenum ($(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$), 0·04 p.p.m.

Zinc, by itself, was used at a concentration of 0·4 p.p.m.

Results

INFLUENCE OF GROWTH FACTORS ON GROWTH

Growth of both strains of Sterile Mycelium and *Rhizoctonia solani* 6T was very poor without added thiamin (table 4). It would appear from table 5 that Sterile Mycelium 2P could, to some extent, overcome its dependence on added thiamin in the presence of trace metals. Although Elarosi (1957), Houston (1939) and Mathew (1953) have recorded increased growth of *Rhizoctonia solani* with added thiamin, most strains, like strain 7P studied here, are not dependent on it.

TABLE 4—Influence of Growth Factors and Trace Elements on Growth

Strain		Yields Dry Mycelium, mg/100 mg Glucose Supplied				
		Medium Supplemented With				
		Nothing	Growth Factors	Thiamin	Trace Elements	Zinc
Sterile Mycelium	1T	1	15	15	21†	20†
	2P	1	15	6	15†	14†
Rhizoctonia sp.	3T	8*	7*	7*	13†	15*†
	4T	21*	18*	19*	26†	28†
	5P	10*	11*	12*	12*†	12†
Rhizoctonia solani	6T	<1	–	19	42†	29†
	7P	26	26	26	26†	26†
Cylindrocarpon radicicola	8T	21	17	–	30	29
	9F	10	6	–	25	24
	10P	10*	10*	–	24	25
	11P	22*	19*	–	25	25
Fusarium culmorum	12P	21	20*	–	25	29
Fusarium sambucinum var. *coeruleum*	13T	7*	11	–	19	19
Fusarium oxysporum	14P	29	24	–	31	31
	15P	32	29*	–	32	33
	16P	13*	9*	–	28	26
	17T	7*	6*	–	14*	14*

– Signifies no determination made.
† Media contained thiamin (1·0 p.p.m.).
* Crop yields recorded for the longer incubation period.

"Thiazole" could be synthesised by the three thiamin-dependent strains but none of them could synthesise the "pyrimidine" moiety efficiently and the addition of "pyrimidine" was necessary for good growth (table 5). A strain of *Corticium (Rhizoctonia) microsclerotia* studied by Mathew (1953) also failed to synthesise "pyrimidine".

TABLE 5—Influence of Thiamin and its Components on Growth

Strain		Yields Dry Mycelium, mg/100 mg Glucose Supplied				
		Medium Supplemented With				
		Nothing	"Thiazole"	"Pyrimidine"	"Thiazole" + "Pyrimidine"	Thiamin
Sterile Mycelium	1T	<1	1	26	25	26
	2P	11	14	28	37	35
Rhizoctonia solani	6T	<1	1	35	37	36

The strains of the *Rhizoctonia* sp., *Cylindrocarpon* and the Fusaria, with the possible exception of *Fusarium sambucinum* var. *coeruleum,* did not require added growth factors.

INFLUENCE OF TRACE ELEMENTS ON GROWTH

Many of the fungi grew better on the addition of trace elements (table 4). With most species, considerable strain variations occurred. With the possible exception of *Rhizoctonia solani* 6T, this stimulation was due solely to zinc.

Stimulation of the growth of *Rhizoctonia solani* by zinc has been previously noted by Steinberg (1950), who also showed the necessity of the trace elements iron, copper, manganese, and molybdenum by the use of highly purified media.

A marked increase in the intensity of the brown pigmentation of all strains of *Cylindrocarpon radicicola* occurred on the addition of zinc. Although the growth of *Fusarium culmorum* and of two strains of *Fusarium oxysporum* was not greatly increased on adding zinc, the pigmentation of these and the other strains of *Fusarium oxysporum* was more intense in its presence.

UTILISATION OF ALIPHATIC CARBON COMPOUNDS

Some of the monosaccharides and other carbohydrates frequently found in nature (Vallentyne 1957) have been tested as substrates for growth of the fungi.

The acids examined here are mainly members of the Krebs' citric acid cycle and might be of common, even though of transitory and limited occurrence in the soil.

Materials and Methods

Media

All media contained basal salts solution and zinc (0·4 p.p.m.). Media for the Sterile Mycelia and *Rhizoctonia solani* 6T also contained thiamin (1·0 p.p.m.). Carbon and nitrogen substrates were added as follows:

Media with Carbohydrates and Alcohols

These media contained sodium nitrate (0·2%). The pH values of the basal media were adjusted before the addition of the carbohydrates and alcohols.

Hemicellulose and cellulose were added at a concentration of 1·0% and the other carbohydrates and alcohols at a concentration of 3·0%. Ethanol was added without sterilisation to the sterilised basal medium. No contamination of uninoculated ethanol medium was seen on incubation for 56 days.

Media with Aliphatic Acids

As high concentrations of aliphatic acids can inhibit the growth of fungi, the acids were added to the present media at the concentration (0·5%) used by Johnson (1957) in similar nutritional studies.

In initial experiments, the media contained sodium nitrate and the acids were neutralised with sodium hydroxide. The pH values of these media became very alkaline, however, during growth. More favourable pH values of the media were maintained by neutralising the acids with ammonia. The media were therefore adjusted to the usual pH values, before sterilisation, with concentrated ammonia which was the only nitrogen compound added. These media would all have contained about the same amounts of nitrogen, *viz.* a mean value of about 0·1% N, which was about two-three times greater than the concentrations of nitrogen present in Raulin-Thom and Czapek-Dox media. It is possible that the ammonium salts in these aliphatic acid media would have had some inhibitory effect on the growth of some of these fungi but the amounts of nitrogen would not be expected to be toxic for most fungi.

"Control" media, without added carbon and nitrogen compounds, were also used.

Periods of Incubation

Cultures on the cellulose medium were incubated for 72 days. On the other media containing carbohydrates and alcohols, the incubation periods used were those listed under General Materials and Methods.

On the aliphatic acid media, cultures of the Fusaria were incubated for 21 days and all other cultures for 28 days.

Estimation of Growth

In most of these media the yields of dry mycelial felts were determined. Growth in media containing the aliphatic acids was often poor. Yields of mycelial felts from the acid media were, therefore, corrected for the slight amount of growth which resulted in the "control" media from the presence of impurities, probably introduced with the inocula.

In the cellulose medium, the mycelial felts could not be separated from residual cellulose. Growth in this medium was estimated by measuring the amount of nitrogen incorporated in the filtered, washed mycelial felts and residual cellulose (table 7). The cellulase activity of some of these culture fluids was also determined.

CHEMICAL TESTS

Determination of Nitrogen

The amount of nitrogen in mycelial felts was determined by the Kjeldahl method. A correction was made for the trace amounts of nitrogen present in cellulose itself and in fungal mycelia grown on the basal salts-nitrate medium without added cellulose. The digestion was made with sulphuric acid (50%, v./v.) containing selenium dioxide (1%), and the ammonia formed distilled in the apparatus of Parnas and Wagner (Niederl and Niederl 1942). The distillate was collected in boric acid (1% solution in ethanol, 20% v./v.) containing bromocresol green-methyl red indicator and titrated with 0·02 N-HCl (Conway and O'Malley 1942).

Determination of Cellulase Activity

The culture filtrates (1·0 ml) produced after growth in the cellulose medium were added to suspensions of cellulose (0·25%) in 9·0 ml succinic acid-sodium borate buffer (0·05 M, pH 5·0). The stoppered flasks were maintained for 72 hr at 50°C and the reducing sugars produced measured by the method of Somogyi (1945).

Results

UTILISATION OF CARBOHYDRATES AND ALCOHOLS

The growth of the Sterile Mycelia and of all strains of the *Rhizoctonia* sp. was usually slow on these media. Maximal yields of *Rhizoctonia solani,* however, usually resulted within the incubation period of 28 days. *Cylindrocarpon radicicola* 10P utilised several carbohydrates slowly but the mycelial yields of the other three strains were usually higher after incubation for 21 days than after 28 days. Growth of the Fusaria was rapid and nearly all substrates gave greater yields after 14 days than after 21 days.

Rhizoctonia sp. 5P and *Fusarium oxysporum* 17T grew much less vigorously than the other strains of these two species.

It can be seen from table 6 that high mean yields of all strains were obtained on xylose, galactose, glucose, and starch. Cellobiose, sucrose, and trehalose were generally utilised well except by some strains of the Sterile Mycelia and Rhizoctoniae.

Ribose was utilised poorly by the non-sporing fungi but utilised well by most non-sporing fungi.

All strains were able to utilise hemicellulose. Crop yields were sometimes low with strains grown on the hemicellulose supplied commercially and it is possible that this material was less readily attacked than the xylan isolated from beech wood.

With the exception of *Rhizoctonia* sp. 5P and *Rhizoctonia solani* 6T, all fungi grew well with pectin. The failure of *Rhizoctonia solani* 6T to grow on this pectin medium could have been due to its acidity, *ca.* pH 4, as in media of initial pH 4·4. Elarosi (1957) found that *Rhizoctonia solani* did not utilise it well. *Rhizoctonia solani* and *Cylindrocarpon radicicola* were found by Elarosi (1958) to possess similar pectic enzymes which differed from the more active Fusaria studied by him.

Inulin, ethanol, and glycerol were generally utilised more readily by the sporing than the non-sporing fungi. These two groups of fungi also differed markedly in their utilisation of mannitol which was the optimal substrate for half of the sporing fungi.

The tussock-grassland strains of the non-sporing fungi, *viz.* Sterile Mycelium 1T, *Rhizoctonia* sp. 3T and *Rhizoctonia solani* 6T, grew well with D-mannose, D-fructose and α-D-melibiose, and poorly with D-arabinose, L-arabinose, dulcitol, and D-sorbitol. The other fungi were not grown on these substrates.

After the incubation periods employed in table 6, the culture fluids of *Rhizoctonia* sp. 5P were respectively pH 5·5 and 4·9 on glucose and sucrose media. In all other media and with all other fungi the pH values of the culture fluids after growth were above pH 6·5. It is clear, therefore, that large amounts of organic acids were not produced as metabolic products from the utilisation of the carbohydrates and alcohols.

With the exception of growth on arabinose, dulcitol and mannitol, the activities of the strains of *Rhizoctonia solani* studied here were similar to those found by Müller (1924), Lilly and Barnett (1953) and Butler (1957) with strains pathogenic to plants.

Simple growth requirements and the ability to use a wide range of compounds as sole sources of carbon have previously been found with varieties of *Fusarium oxysporum* pathogenic for plants, e.g., by Moore and Chupp (1952) and Rishbeth (1957).

Utilisation of Cellulose

All strains could utilise cellulose as sole source of carbon and energy (table 7).

The Rhizoctoniae generally grew well and the cellulose fibres were almost completely disintegrated by both strains of *Rhizoctonia solani*. The comparatively low nitrogen content of mycelial felts of *Rhizoctonia solani* 6T could have been due to lysis of cell material and the conversion of some of its nitrogen to water-soluble forms. An indication of the cellulase activity of some of the culture fluids of the non-sporing fungi is given by the data in table 8 which confirmed the production of soluble cellulolytic enzymes by these strains. It is of interest to note that a strain of each of two recognised cellulolytic fungi, *Humicola grisea* and a *Chaetomium* sp., isolated from tussock-grassland soil, showed similar cellulase activity to these cultures when tested under the same conditions.

TABLE 6—Utilisation of Carbohydrates and Alcohols

	Fungus		Yields: % of Yield on Optimal Substrate. Substrate:														Yields Dry Mycelium, mg/100 mg Carbohydrate or Alcohol Supplied	
			Carbohydrates											Alcohols				
			D-Ribose	D-Xylose	D-Galactose	D-Glucose	D-Cellobiose	Sucrose	α-α′-D-Trehalose	Hemicellulose	Inulin	Pectin	Starch	Ethanol	Glycerol	Mannitol	Yields on Optimal Substrate	Mean Yields (Coefficients of Variation, %)
Non-sporing fungi	Sterile Mycelium	1T	0	95	100	95	93	13	41*	59*†	70	75*	70	16	43*	13*	*44*	*24·7* (63)
		2P	7	100	82	100	93	7*	35*	14*	25	53	84	28	21*	11*	*57*	*26·8* (79)
	Rhizoctonia sp.	3T	0	84	100	53	28*	63	74	32†	81	53*	84	19	60*	5	*57*	*29·2* (62)
		4T	4	89	96	89	100*	98	90	29	77	61	83	37	54	31	*52*	*34·8* (47)
		5P	14*	76	95	60	52*	79	81	33	10*	26*	100	7*	79	16*	*42*	*21·9* (64)
	Rhizoctonia solani	6T	0	81	98	87	100	100	81	40†	75	0	83	8	30	4	*53*	*29·8* (72)
		7P	4	78	100	81	89	89	78	30	9*	67	83	24*	30*	39*	*54*	*30·9* (58)
	Cylindrocarpon radicicola	8T	92	94	92	92	92	100*	86	36†	94	67	92	28	67	94	*36*	*28·9* (28)
		9F	78	83	86	75	89	89	78	36	83*	72	78	56	92	100	*36*	*28·1* (21)
		10F	13*	92*	90	82	95*	100	92*	29	90	69*	84	34*	100	95	*38*	*28·9* (35)
		11P	79	90	93	81	83	93	79	29	48*	79	79	40	100	98	*42*	*32·1* (29)
	Fusarium culmorum	12P	90	95	80	80	93*	88	83	43†	93	80	93	68*	13*	100	*40*	*31·4* (30)

Sporing fungi	*Fusarium sambucium* var. *coeruleum*	13T	81*	78	81	66	84	78	81	16	78	63	81	78	100*	100*	*32*	*24·4* (27)
	Fusarium oxysporum	14P	79*	95	87	92	97	97	84	42†	90	82	84	26	87*	100	*38*	*31·0* (26)
		15P	92	100	95	95	97	100	89	41†	95	95	84	54*	70*	89	*37*	*31·6* (21)
		16P	82	92	87	82	100	87	87	13	79	79	90	82	90	92	*38*	*30·9* (25)
		17T	84	55	74	61	65	65	78	19	81	71	84	92	100	100	*31*	*22·4* (28)
Yields (as percentage of yields on optimal substrates) of all strains on each substrate; means ± standard deviations.	Non-sporing fungi		4 ± 5	86 ± 9	96 ± 6	81 ± 18	79 ± 28	64 ± 39	69 ± 22	34 ± 14	50 ± 33	48 ± 26	84 ± 9	20 ± 11	45 ± 20	17 ± 13		
	Sporing fungi		77 ± 23	87 ± 11	87 ± 7	81 ± 11	90 ± 10	90 ± 11	84 ± 5	30 ± 11	83 ± 14	76 ± 9	86 ± 5	56 ± 24	82 ± 27	97 ± 4		

† Xylan supplied by Mr I. R. C. McDonald, Dominion Laboratory, Wellington. The other samples of hemicellulose were supplied by L. Light and Co. Ltd.

* Crop yields recorded for the longer incubation period.

TABLE 7—Utilisation of Cellulose

Non-sporing Fungi		Nitrogen Content of Mycelial Felts (mg/flask)	Sporing Fungi		Nitrogen Content of Mycelial Felts (mg/flask)
Sterile Mycelium	1T	1.83	*Cylindrocarpon radicicola*	8T	1.52
	2P	1.56		9F	1.63
				10P	2.63
				11P	2.04
Rhizoctonia sp.	3T	1.13			
	4T	1.27			
	5P	2.00	*Fusarium culmorum*	12P	1.83
Rhizoctonia solani	6T	1.00	*Fusarium sambucinum* var. *coeruleum*	13T	0.95
	7P	4.52			
			Fusarium oxysporum	14P	3.30
				15P	2.52
				16P	1.95
				17T	2.29
				18P	3.16
				19P	3.98

Uninoculated media contained 6.59 mg N/flask.

TABLE 8—Cellulase Activity of Culture Filtrates

Strain		Reducing Sugars Produced, Calculated as Glucose, mg/ml Culture Filtrate
Sterile Mycelium	1T	1.72
Rhizoctonia sp.	3T	3.93
Rhizoctonia solani	6T	0.82

Matsumoto (1921) found that *Rhizoctonia solani* utilised cellulose but it has normally been regarded as a weakly cellulolytic organism, *e.g.* by Müller (1924) and Blair (1943). Kohlmeyer (1956), however, found the production of large quantities of cellulase by a strain of *Rhizoctonia solani* isolated from a potato sprout and it is apparent that the strains studied here also have marked cellulolytic ability.

All strains of *Cylindrocarpon radicicola* grew well but did not completely hydrolyse the cellulose.

Moderate cellulose utilisation by *Fusarium culmorum* has previously been reported by Jensen (1931) and Venkata Ram (1957). Venkata Ram (1957) also found that the cellulolytic activity of *Fusarium sambucinum* var. *coeruleum* was low and the activity of *Fusarium oxysporum* was high. In the present experiments, the cellulose fibres were nearly completely disintegrated by all strains of *Fusarium oxysporum*.

Utilisation of Aliphatic Acids

The Sterile Mycelia and Rhizoctoniae, in general, grew poorly with these substrates and crop yields were low (table 9). Sterile Mycelium 1T and *Rhizoctonia* sp. 4T and 5P failed to grow on some of the media. All the aliphatic acids were utilised to some extent by *Rhizoctonia solani,* with the exception of acetic acid by strain 6T. In a medium containing sodium nitrate, however, *Rhizoctonia solani* 6T and Sterile Mycelium 1T could use acetate when present as the sodium salt.

All strains of *Cylindrocarpon radicicola* grew well with these acids although some strain variations occurred.

Fusarium culmorum grew well with most acids. High crop yields with all acids were obtained with *Fusarium sambucinum* var. *coeruleum* and with all strains of *Fusarium oxysporum.*

Utilisation of Aromatic Carbon Compounds

Many aromatic compounds have been isolated from the decomposition of lignin and the ability of soil fungi to utilise some of these simple molecules could be a guide to their ability to use more complex degradation products of lignin in nature (Henderson and Farmer 1955). Some of these lignin oxidation products have been used in the present work.

When wood-rotting fungi are grown in gallic acid or tannic acid media, most strains associated with white rot, *i.e.* lignin oxidisers, cause a browning of the medium, whereas most of those associated with brown rot, *i.e.* cellulose decomposers, do not give a brown colour (Davidson *et al.* 1938). The test is not entirely specific (Etheridge 1957) but has generally been confirmed with wood-rotting fungi. This test has been used in the present work as it could indicate the ability of soil fungi to utilise lignin.

Materials and Methods

Media

All media contained basal salts solution, sodium nitrate (0·2%) and zinc (0·4 p.p.m.). Media for the Sterile Mycelia and Rhizoctoniae contained also thiamin (1·0 p.p.m.).

p-Hydroxybenzaldehyde, ferulic acid, and vanillin were dissolved in sterile water and added to the separately sterilised basal medium. Their concentration (0·01%) in the media was low as they can inhibit growth of some fungi at high concentrations (Henderson and Farmer 1955).

Gallic acid (0·25%) and tannic acid (0·25%) were added, after separate sterilisation, to the molten basal medium containing agar (1·5%).

Periods of Incubation

The liquid cultures were incubated for 28 days and cultures on the gallic acid and tannic acid media were incubated for 7 days.

Estimation of Growth

Because of the very low yields of mycelial felts, growth on the liquid media was estimated by chemical tests.

TABLE 9—Utilisation of Aliphatic Acids

Strain		Yields: Percentage of Yield on Optimal Substrate. Acid Substrate:								Yields Dry Mycelium mg/100 mg Acid Supplied	
		Acetic	Citric	Fumaric	α-Keto-glutaric	Malic	Succinic	Lactic	Tartaric	Yields on Optimal Substrate	Mean Yields (Coefficients of Variation,%)
Sterile Mycelium	1T	0	100	88	100	0	100	0	0	*8*	*3·9* (107)
	2P	30	60	40	80	40	100	80	10	*10*	*5·5* (56)
Rhizoctonia sp.	4T	40	0	0	0	40	60	100	20	*5*	*1·6* (113)
	5P	100	0	0	17	17	17	17	0	*6*	*1·3* (153)
Rhizoctonia solani	6T	0	57	43	86	86	100	43	14	*7*	*3·8* (90)
	7P	40	50	60	80	50	100	30	10	*10*	*5·3* (53)
Cylindrocarpon radicicola	8T	69	54	84	62	84	100	54	65	*26*	*18·6* (23)
	9F	76	38	48	48	57	71	100	43	*21*	*12·6* (35)
	10P	57	43	91	78	52	74	100	57	*23*	*15·9* (29)
	11P	100	77	86	64	64	86	86	91	*22*	*18·0* (16)
Fusarium culmorum	12P	64	21	57	14	50	86	100	0	*27*	*13·6* (71)
Fusarium sambucinum var. *coeruleum*	13T	100	50	90	80	90	100	50	70	*20*	*15·8* (26)
Fusarium oxysporum	14P	87	67	87	80	80	100	93	67	*30*	*24·8* (16)
	15P	81	50	81	100	75	94	94	63	*32*	*25·5* (21)
	16P	81	63	75	94	75	100	94	56	*32*	*25·5* (20)
	17T	90	90	100	70	70	90	90	70	*20*	*16·8* (14)

The diameters of the mycelial colonies, corrected for the inoculum disks, were measured on the gallic acid and tannic acid media.

Chemical Tests

Uninoculated media containing p-hydroxybenzaldehyde and vanillin gave a dense orange precipitate with an aqueous solution of 2 : 4-dinitrophenylhydrazine hydrochloride (Brady's reagent) whereas media in which these substrates had been completely oxidised no longer gave a precipitate.

Utilisation of ferulic acid was estimated qualitatively by the amount of mycelial growth and, or, the development of a pale brown-yellow colour in the culture fluids. Where growth was slight, the addition of ammonia (2 drops) to these fluids (5 ml) caused a deepening of colour, due possibly to its reaction with quinonoid metabolic products. Ferulic acid and vanillic acid, a possible oxidation product, do not form a pigment with ammonia.

Results

Growth of the Sterile Mycelia and Rhizoctoniae was generally very slight in media containing p-hydroxybenzaldehyde, ferulic acid and vanillin (table 10). Vanillin initially appeared to inhibit growth. p-Hydroxybenzaldehyde was completely utilised by Sterile Mycelium 1T. In all other cases, however, these substrates were not completely oxidised by these non-sporing fungi. The yellow-brown culture fluids from the ferulic acid medium gave a positive reaction with ammonia and also a slight precipitate with Brady's reagent indicating that some metabolism of the acid had occurred.

All strains of *Cylindocarpon* and the Fusaria grew well on the above compounds. The substrates were completely oxidised as the culture fluids did not react with Brady's reagent or ammonia.

The strains of non-sporing fungi differed considerably in their utilisation of gallic acid and tannic acid but their growth, particularly on the gallic acid medium, was often very poor (table 10). *Rhizoctonia* sp. 4T and *Rhizoctonia solani* 7P, however, grew vigorously with the production of much pigment in both media. All strains of *Cylindrocarpon* and the Fusaria grew well on these media but pigment production varied with strain and no pigment at all was produced by the Fusaria on the gallic acid medium. As there seems to be no correlation between pigment production on these media and growth on the other compounds tested, it would appear that no reliance should be placed on pigment production on gallic acid and tannic acid media as a means of assessing the ability of these soil fungi to utilise lignin degradation compounds in nature. Henderson and Farmer (1955) also concluded that there was probably no correlation between the ability of soil fungi to oxidise the simple aromatic compounds and to oxidise tannic acid.

Utilisation of Nitrogen Compounds

The amounts of inorganic nitrogen compounds in soil are usually small and the ability of an organism to use organic nitrogen compounds for its nitrogen requirements could be of considerable ecological advantage. Growth of these fungi has therefore been studied on some of these compounds.

TABLE 10—Utilisation of Aromatic Compounds

Strain		Qualitative Assessment of Growth With†			Diameters of Colonies (mm) on:††	
		p-Hydroxy-benzaldehyde	Ferulic Acid	Vanillin	Gallic Acid	Tannic Acid
Sterile Mycelium	1T	++	+	+	2 LB	24 B
	2P	+	+	+	1 LB	26 B
Rhizoctonia sp.	4T	+	+	+	40 B	48 B
	5P	+	+	+	13	7 LB
Rhizoctonia solani	6T	+	+	+	7 LB	28 B
	7P	+	+	+	84 B	84 B
Cylindrocarpon radicicola	8T	++	++	++	54 LB	34 B
	9F	++	++	++	46	39
	10P	++	++	++	24 LB	32
	11P	++	++	++	37	37
Fusarium culmorum	12P	++	++	++	59	27 B
Fusarium sambucinum var. *coeruleum*	13T	++	++	++	32	38 LB
Fusarium oxysporum	14P	++	++	++	45	34 B
	15P	++	++	++	40	31 B
	16P	++	++	++	60	64
	17T	++	++	++	55	46

† + signifies slight growth and, or, some utilisation of substrate;
++ signifies growth and complete utilisation of substrate.

†† Changes in the media after growth are signified as follows:
B = brown-dark-brown pigmentation;
LB = light brown pigmentation;
No symbol = no pigmentation of the medium.

Some of the commercially available proteins and nucleic acids have been used as sole sources of nitrogen. Although these compounds would be denatured during sterilisation of the media it is thought that the ability of the fungi to use them would provide an indication of the proteolytic ability of the fungi in their natural habitats.

Most of the pyrimidines and purines isolated from soil (Vallentyne 1957) have also been employed as sole sources of nitrogen for these fungi.

Materials and Methods

MEDIA

Media With Inorganic Nitrogen Compounds and Amino Acids (Table 11)

All these media contained basal salts solution and glucose (5·0%) and were used in 50 ml portions in 250 ml conical flasks.

Non-sporing Fungi

The media contained $NaNO_3$ (0·2%), NH_4NO_3 (0·09%), casamino acids (0·5%) or peptone (1·0%). The sodium and ammonium nitrate media contained thiamin (1·0 p.p.m.).

Sporing Fungi

Nitrogen compounds were added at a concentration of 0·0334% N as follows: $NaNO_3$ (0·2%), $(NH_4)_2HPO_4$ (0·16%), casamino acids (0·35%), or peptone (0·22%).

Media With Proteins, Nucleic Acids, Pyrimidines and Purines (Table 12)

The media contained basal salts solution, glucose (3%), zinc (0·4 p.p.m.) and nitrogen substrate (0·0167% N, *i.e.* equivalent to 0·1% $NaNO_3$). Media containing the partly-insoluble substrates guanine, albumen (from blood) and gluten, were boiled and cooled before adjusting to the usual pH values.

Media for the Sterile Mycelia and *Rhizoctonia solani* 6T contained thiamin (1·0 p.p.m.).

"Control" media contained no added nitrogen compounds.

Media With Egg White and Gelatin (Table 13)

Fresh egg white (about 66% v./v.), obtained under aseptic conditions, was added to basal salts solution, glucose (1%) and zinc (0·4 p.p.m.). Slopes of the medium were prepared in McCartney bottles and the medium solidified and sterilised by steaming for 30 min. on two successive days.

Aqueous gelatin (10%) was sterilised by steaming for 30 min. on three successive days in McCartney bottles.

Cultural Conditions

Gelatin cultures were incubated at 20°C and egg white cultures at the usual temperatures for 28 days.

Cultures on all other media were incubated as usual.

Estimation of Growth

Utilisation of the solidified egg white was assessed by shaking the bottles vigorously. A soft mush was produced by the most active fungi.

The depth of liquefaction was measured in the gelatin cultures.

Dry weights of mycelial felts were determined in the other experiments. Yields on the protein, nucleic acid, pyrimidine and purine media were corrected by subtracting the slight yields obtained on the "control" media without added nitrogen compounds.

RESULTS

UTILISATION OF INORGANIC NITROGEN COMPOUNDS AND AMINO ACIDS

As the media for the non-sporing and sporing fungi contained different amounts of ammonium compounds, amino acids and peptone, results from these two groups of fungi cannot be quantitatively compared.

All cultures were able to use nitrate and yields of mycelium were generally satisfactory (table 11). The pH values of the nitrate media usually rose during growth.

Yields were often low in media containing ammonium compounds due probably to the development of acidity. The culture fluid after growth of *Rhizoctonia solani* was at pH 6·5 but all other culture fluids were at pH 3·5 or less.

Although casamino acids were utilised by all the fungi, it is clear that preformed amino acids were not essential for growth.

TABLE 11—Utilisation of Inorganic Nitrogen Compounds and Amino Acids

	Fungus		Yields Dry Mycelium, mg/100 Glucose Supplied			
			Nitrogen Substrate:			
			Sodium Nitrate	Ammonium Salt†	Casamino Acids†	Peptone†
Non-sporing fungi	Sterile Mycelium	1T	15	2	3	29
		2P	6	2	2	14
	Rhizoctonia sp.	3T	7*	1	15*	44*
		4T	19*	16*	15	24
		5P	12*	3	6	29*
	Rhizoctonia solani	6T	19	–	2	42
		7P	26	21	29	38
Sporing fungi	*Cylindrocarpon radicicola*	8T	21	10	25	31
		9F	10	9	8	25*
		10P	10*	8	12	13
		11P	22*	18	19*	18
	Fusarium culmorum	12P	21	6	25	16
	Fusarium sambucinum var. *coeruleum*	13T	7*	7	6*	10
	Fusarium oxysporum	14P	29	17*	23	20
		15P	32	19	29	21
		16P	13*	5*	8	10
		17T	7*	7	7	7*

– Signifies no determination made.

* Crop yields recorded for the longer incubation period.

† Different concentrations of these compounds were present in media for the non-sporing fungi and sporing fungi.

All fungi grew readily on the peptone medium. Growth in the casamino acid and peptone media was usually accompanied by an initial drop in the pH of the culture fluids.

Utilisation of Proteins and Nucleic Acids

In general, the proteins and nucleic acids supported good growth of all strains (table 12). Yields of mycelium were slightly, but consistently, higher on gluten than on casein. The lower yields obtained with albumen (from blood) can probably be explained by an error in the preparation of this medium as the initial pH was 4·7. Sterile Mycelium 1T grew slowly and mainly by means of submerged hyphae on the nucleic acid media. On the other media it produced surface felts with little submerged growth. Moderate growth of *Fusarium oxysporum* in a nucleic acid medium has been previously found by Gottlieb (1946).

On the egg white medium, the Sterile Mycelia and Rhizoctoniae grew moderately well but none of these strains possessed marked ability to break down this substrate (table 13). Growth of all strains of *Cylindrocarpon radicicola* was vigorous and extensive degradation of the medium occurred. *Fusarium culmorum*, *Fusarium sambucinum* var. *coeruleum* and *Fusarium oxysporum* 16P showed little ability to utilise egg white but the other three strains of *Fusarium oxysporum* grew vigorously with extensive degradation of the medium.

Gelatin was liquefied in a stratiform manner by all the fungi. The correlation between gelatin liquefaction and general ability to utilise proteins was poor as the Sterile Mycelia were the strongest liquefiers but did not give the highest crop yields with the other proteins. The pathogenic strains of *Rhizoctonia solani* studied by Matsumoto (1921) all liquefied gelatin.

Utilisation of Pyrimidines and Purines

Cytosine or uracil was utilised by the *Rhizoctonia* sp. but pyrimidines were generally not utilised by the Sterile Mycelia and Rhizoctoniae. Although hypoxanthine was sometimes poorly utilised, the other purines generally supported good yields of the non-sporing fungi (table 12).

Cytosine and thymine were not utilised by *Cylindrocarpon radicicola* but all strains grew well with uracil and with the purines.

Fusarium culmorum grew moderately well with uracil and *Fusarium sambucinum* var. *coeruleum* could use cytosine to a slight extent. Otherwise, the Fusaria could not utilise the pyrimidines. All purines, however, were utilised by all the Fusaria.

All fungi grew well with uric acid as nitrogen substrate.

Metabolic Studies

The amounts of glucose utilised by some strains of the fungi were determined to assess the efficiency of its assimilation for cell synthesis. Culture fluids and mycelial felts were also examined to estimate the amounts of non-volatile metabolic products extractable in organic solvents.

TABLE 12—Utilisation of Proteins, Nucleic Acids, Pyrimidines, and Purines

Strain		Yields: % of Yield on Optimal Substrate. Substrate:														Yields Dry Mycelium, mg/100 mg Glucose Supplied	
		Nitrate	Proteins			Nucleic Acids From:		Pyrimidines			Purines			Uric Acid		Yields on Optimal Substrate	Mean Yields (Coefficients of Variation, %)
			Albumen (From Blood)	Casein	Gluten	Thymus	Yeast	Cytosine	Thymine	Uracil	Adenine	Guanine	Hypoxanthine				
Sterile Mycelium	1T	50	50	63	100	40*	54*	0	0	0	25*	25*	2	56		*48*	*17·2* (61)
	2P	100	37	46	76*	68	70	0	0	0	59	59	27	70		*37*	*17·5* (69)
Rhizoctonia sp.	4T	70	50	97	100	97	98	0	0	44*	81	39*	61*	66		*64*	*38·9* (66)
	5P	52	31	72	100	65	69*	17*	0	0	55*	34*	3*	62*		*29*	*12·6* (73)
Rhizoctonia solani	6T	42	28	62	91*	85	100	0	0	0	35*	17	40*	49		*65*	*26·9* (82)
	7P	56	66	69	87	93	100	0	2	0	69	42	5	61		*59*	*29·5* (74)
Cylindrocarpon radicicola	8T	79	63	85	100	100	94	0	0	67*	88	67	88	85		*33*	*23·2* (48)
	9F	75	55	90	100	95	100	0	0	93	93	83	93	88		*40*	*29·6* (47)
	10P	67	62	74	87	97	100	0	0	74*	74	77	74	79		*39*	*26·0* (47)
	11P	85	80	93	98	98	100	0	0	83	88	93	90*	98		*40*	*30·9* (45)
Fusarium culmorum	12P	79*	32*	71	100	97	84	3	0	29*	50	66*	87*	84		*38*	*22·9* (57)
Fusarium sambucinum var. *coeruleum*	13T	74	46*	74*	97	97	100	11*	0	0	74	68*	80	80		*35*	*21·6* (58)
Fusarium oxysporum	14P	70	54	76	87	100	100	0	0	0	84	57	87	78		*37*	*22·5* (61)
	15P	74	45	71	90	86	100	0	0	0	76	45*	76	76		*42*	*23·9* (70)
	16P	68	48	71	88	98	100	0	0	0	83	63*	83*	78		*41*	*24·6* (61)
	17T	34	48*	66*	100	66	90	0	0	0	55	21*	55	48		*29*	*13·0* (73)

* Crop yield recorded for the longer incubation period.

TABLE 13—Utilisation of Egg White and Gelatin

Strain		Utilisation of Egg White	Gelatin Liquefaction, Depth (mm)
Sterile Mycelium	1T	+	17
	2P	+	19
Rhizoctonia sp.	4T	+	9
	5P	+	14
Rhizoctonia solani	6T	+	16
	7P	+	12
Cylindricarpon radicicola	8T	+++	14
	9F	+++	9
	10P	+++	14
	11P	+++	9
Fusarium culmorum	12P	+	10
Fusarium sambucinum var. *coeruleum*	13T	+	15
Fusarium oxysporum	14P	+++	10
	15P	+++	10
	16P	+	8
	17T	+++	13

+ Signifies poor growth and decomposition of medium.
+++ Signifies good growth and decomposition of medium.

Materials and Methods

MEDIA

Media for Non-sporing Fungi

Czapek-Dox and Raulin-Thom media were supplemented with trace elements at the concentrations used previously in the media for trace element studies. Media for growth of the Sterile Mycelia and *Rhizoctonia solani* also contained thiamin (1·0 p.p.m.). The media (150 ml) were contained in 500 ml conical flasks.

Media for Sporing Fungi

For growth of *Cylindrocarpon radicicola* the initial pH values of Czapek-Dox and Raulin-Thom media were 4·0 and 5·0. For the Fusaria, these media were adjusted to the usual value, pH 6·0. The media (350 ml) were contained in 1 1 conical flasks.

ESTIMATION OF EFFICIENCY OF GLUCOSE ASSIMILATION

Residual glucose was determined by the method of Somogyi (1945) in the fluids of cultures harvested at about weekly intervals, commencing at the first incubation periods listed in table 14. The efficiency of glucose assimilation was calculated by dividing the weight of dry mycelium by the amount of glucose consumed and multiplying by 100.

ESTIMATION OF SOME NON-VOLATILE METABOLIC PRODUCTS IN CULTURE FLUIDS

Culture fluids were tested with an aqueous solution of ferric chloride to detect phenolic compounds or other compounds giving a deep colour with this reagent.

Cultures of Sterile Mycelium, Rhizoctoniae and *Cylindrocarpon* were harvested at about weekly intervals, commencing at the first incubation periods listed in table 14, and the fluids separated from the mycelial felts and stored in the refrigerator. Equal amounts of the culture fluids from each harvest were then combined, adjusted to pH 4, and 500 ml portions extracted with three 100 ml portions of ethyl acetate. The extracts were dried over anhydrous sodium sulphate and the solvent removed at about 40°C *in vacuo*.

The culture fluids of the Fusaria were harvested only once, after 14 days, adjusted to pH 4, and extracted three times with diethyl ether.

Yields of extracted material were expressed as mg/100 ml combined culture fluids.

ESTIMATION OF NON-VOLATILE METABOLIC PRODUCTS EXTRACTED BY ORGANIC SOLVENTS FROM MYCELIAL FELTS

Cultures of the fungi were harvested at about weekly intervals, commencing at the first incubation periods listed in table 14. The mycelial felts were separated by filtration, washed with water, dried for 72 hr at 50°C and coarsely ground with a pestle and mortar. The felts of the Fusaria from the different harvests were extracted separately but the felts of the other strains from the different harvests were combined for extraction. The felts were extracted for about 25 hr with each solvent in a Soxhlet apparatus successively with light petroleum (b.p. 40°–60°C), diethyl ether, and ethanol (90% v./v.). Solvents were removed, ethanol *in vacuo*, and all extracts were finally dried *in vacuo* at about 40°–50°C.

Yields of extracted material were expressed as grammes/100 grammes dry mycelial felts.

RESULTS

EFFICIENCY OF GLUCOSE ASSIMILATION

The values for the efficiency of glucose assimilation were generally similar in each medium. Sterile Mycelium 1T, however, grew less efficiently in the Raulin-Thom medium. In general, the values were highest in the early stages of growth. A considerable amount of the glucose was converted into cell material by all strains and especially by the Sterile Mycelium and the Rhizoctoniae.

A very high yield of cell material from glucose has been previously found for a strain of *Rhizoctonia solani* by Lilly and Barnett (1953). The values for the present cultures of Fusaria were, however, much higher than the values of 8–15% found by Birkinshaw *et al.* (1931) after growth of *Fusarium sambucinum* and *Fusarium oxysporum* for 45 days under similar cultural conditions. The respective values of 48·5% and 33% found by Steinberg (1948) after growth of *Rhizoctonia solani* and *Fusarium oxysporum* on a sucrose medium were almost the same as the present results from glucose. It would, therefore, appear that the efficiency of carbohydrate assimilation varies considerably with the species of fungus and also with cultural conditions.

Metabolic Products in Culture Fluids

In the Czapek-Dox media, there was no evidence of acid production by any of the fungi on examination at weekly intervals (table 14). In the Raulin-Thom media, the pH usually fell considerably due probably to the acidity resulting from the utilisation of ammonium nitrogen.

None of the culture fluids gave a colour with ferric chloride.

Low yields of pale yellow gums were obtained on extraction (table 14). The extracts from the culture fluids of *Fusarium oxysporum* contained a little red pigment. None of the gums crystallised on standing for several weeks and they were not further examined.

Metabolic Products in Mycelial Felts

Sterile Mycelium 1T and the Rhizoctoniae did not synthesise large amounts of fatty material soluble in light petroleum and diethyl ether (table 14). Large amounts of fatty material were, however, produced by *Cylindrocarpon radicicola* and the Fusaria. A little orange-red pigment was present in the extracts from *Fusarium oxysporum*. Similar yields of fatty material were obtained from each strain of the Fusaria and at each incubation period. Nord and Mull (1945) have previously found that large amounts of fat were produced by Fusaria on all their culture media.

The yields of material extracted with ethanol from the Sterile Mycelium were low and yields from the Rhizoctoniae varied considerably. High yields were obtained from *Cylindrocarpon* and the Fusaria. A little pigment was extracted from the Fusaria. The ethanol extracts of all fungi were chiefly brown, viscous gums which deposited some solid material on standing for several weeks. An examination was made of this material from the Rhizoctoniae and *Cylindrocarpon* extracts. The solid deposits were washed with a little methanol and recrystallised from methanol yielding mannitol (yield: about 5–10% of the weight of ethanol extracts), m.p. 166°–167°C, undepressed on admixture with the authentic compound of the same melting point. Identity of this frequently-encountered mould product was confirmed by conversion to the acetate, m.p. 120°–121°C, undepressed on admixture with authentic mannitol hexaacetate, m.p. 121° -122°C. No other compounds were isolated from these deposits.

TABLE 14—Some Properties Associated With Growth in Glucose Media

Strain		Medium†	Incubation Periods, Days	pH of Culture Fluids; Range Over Growth Periods	Residual Glucose, g/100 ml Culture Fluid After Final Incubation Period	Efficiency of Glucose Assimilation; Mean values %, With Ranges of Weekly Values in Parentheses	Yields of Extracts From Culture Fluids; mg/100 ml Culture Fluid	Yields of Extracts From Mycelium; g/100g Dry Mycelium Extracted Successively With	
								Light Petroleum and Diethyl Ether	Ethanol
Sterile Mycelium	1T	CD	14–42	5·5–6·5	1·4	44 (58–36)	8	3·0	8·0
		RT	14–42	3·6–4·9	1·9	33 (39–29)	6	1·4	8·7
Rhizoctonia sp.	3T	CD	14–42	7·2–7·7	2·1	41 (49–33)	5	0·8	22·9
		RT	14–42	3·5–4·1	1·6	44 (52–37)	2	3·5	12·5
Rhizoctonia solani	6T	CD	14–42	7·3–7·8	1·8	47 (55-43)	2	1·1	9·3
		RT	14–42	3·5–4·1	2·0	45 (55–40)	2	1·6	14·6
Cylindrocarpon radicicola	8T	CD	7–42	5·2–6·4	0·1	34††	6	14·2	23·7
		RT	7–42	3·9–6·4	–	–	6	20·9	14·5
Fusarium culmorum	12P	CD	7–21	7·3–8·5	2·1	24 (25–24)‡	3	23·0	23·5
		RT	7–21	7·6–8·1	0·2	27 (34–24)	–	20·7	21·7
Fusarium oxysporum	14P	CD	7–25	7·3–8·4	0·2	28 (34–24)	13	20·0	17·5
		RT	7–17	4·5–8·9	0·1	30 (31–29)	–	22·2	23·5
Fusarium oxysporum	15P	CD	7–25	7·4–8·4	0·8	29 (32–27)	16	23·9	19·6
		RT	7–17	4·9–8·9	0·6	30 (31–29)	–	20·0	21·3

† CD signifies Czapek-Dox and RT signifies Raulin-Thom basal media. Media for strains 1T, 3T, and 6T contained trace metals and media for strains 1T and 6T also contained thiamin. †† Signifies value for 28 days only. ‡Signifies no value for 7 days.
– Signifies no determination made.

Antibiotic Activities

Materials and Methods

Test Organisms

Bacterium coli Bl.2., *Staphylococcus aureus* P.D.D. *strain* B.5.4. and *Rhizobium trifolii* P.D.D. N.Z. 27 were kindly supplied by Dr T. R. Vernon and Mr A. Hastings, Plant Diseases Division, Auckland, and a strain of *Bacillus cereus* var. *mycoides* by Dr J. D. Stout, Soil Bureau, Wellington. *Botrytis allii* Munn. B.R.L. No. 38 was kindly provided by Dr P. W. Brian, Akers Research Laboratories. I.C.I. Ltd., Welwyn, England.

Growth Inhibition Tests

The fungi were grown in 100 ml conical flasks with 20 ml medium composed of basal salts, sodium nitrate (0·2%), glucose (3%) and zinc (0·4 p.p.m.). Media for the Sterile Mycelia and *Rhizoctonia solani* also contained thiamin (1·0 p.p.m.). The cultures were harvested after the following incubation periods:

Sterile Mycelia and Rhizoctoniae, 28 and 56 days;

Cylindrocarpon radicicola, 28 days;

Fusaria, 7, 14, and 21 days.

The culture fluids were decanted from the mycelial felts and the fluids from strains 1T, 5T, 6T, 8T, 12P, 14P, and 15P sterilised by Seitz filtration. The culture fluids of the other strains were stored at — 20°C and were not filtered before use.

The culture fluids were tested, at a concentration of 1 in 10 in a tryptone (1%)-glucose (0·5%) medium, against *Bacterium coli, Staphylococcus aureus* and *Bacillus cereus* var. *mycoides*. The cultures were incubated for 24 hr at room temperature. Their activity against *Rhizobium trifolii* and *Botrytis allii* was determined by adding the culture fluid (0·1–0·2 ml) to a cavity cut in a plate of potato-glucose agar and placing an inoculum of the test organism about 3 mm from the edge of this cavity. Plates were incubated for 2–3 days and any inhibition of growth of the test organisms noted.

Spore Germination Tests

The fungi were grown under the conditions described in the previous section on Metabolic Studies for the following periods:

Sterile Mycelia and Rhizoctoniae, 14, 21, and 42 days;

Cylindrocarpon radicicola, 10 and 21 days;

Fusaria, 14 and 17 days.

The culture fluids of the different strains were harvested and stored as above. These fluids were kindly tested by Dr R. H. Thornton for their ability to inhibit, at a concentration of 1 in 3, the germination of spores of *Botrytis allii*, using the method and media described by Brian and Hemming (1945).

Results

No antibiotic activity was shown against any of the bacteria listed in table 15. Some inhibition of the growth of the mycelium of *Botrytis allii* was caused by the culture fluid of *Cylindrocarpon radicicola* 9F, but no other strain prevented growth of this fungus. Some of the culture fluids of the Sterile Mycelium, *Cylindrocarpon,* and *Fusarium oxysporum,* however, caused inhibition of the germination of spores of *Botrytis allii* (table 15). The inhibitors appeared to be present in only low amounts and their concentration usually depended on the nature of the culture medium.

TABLE 15—Antibiotic Activities of Culture Fluids

Strain		Activity Against Growth of					Inhibition of Germination of Spores (% Inhibition) of *Botrytis allii* by Culture Fluids from	
		Staphylococcus aureus	*Bacillus cereus* var. *mycoides*	*Bacterium coli*	*Rhizobium trifolii*	*Botrytis allii*	Czapek-Dox Media	Raulin-Thom Media
Sterile Mycelium	1T	0	–	0	0	0	†(30–40)	†(30–40)
	2P	0	–	0	–	0	–	–
Rhizoctonia sp.	3T	0	–	0	0	0	0	0
	4T	0	–	0	–	0	–	0
	5P	0	–	0	–	0	–	–
Rhizoctonia solani	6T	0	–	0	0	0	0	0
	7P	0	–	0	–	0	–	–
Cylindrocarpon radicicola	8T	0	–	0	–	0	†0 (50)	0
	9F	0	0	0	–	0	–	–
	10P	0	0	0	–	0	–	–
	11P	0	0	0	–	0	–	–
Fusarium culmorum	12P	0	–	0	0	0	0	0
Fusarium sambucinum var. *coeruleum*	13T	0	0	0	–	0	–	–
Fusarium oxysporum	14P	0	–	0	0	0	0	†(60–100)
	15P	0	–	0	0	0	0	†(10–50)
	16P	0	0	0	–	0	–	–
	17T	0	0	0	–	0	–	–

– Signifies no determination made.

0 Signifies no inhibition against test organism

† Signifies inhibition against test organism.

†0 Signifies no inhibition with 10-day culture fluid, but 50% inhibition with 21-day culture fluid.

A failure of *Rhizoctonia solani* to produce antibiotic compounds has been noted before by Butler (1957).

Arnstein *et al.* (1946) and Lacey (1950) found that, in general, *Fusarium oxysporum* was inactive against *Staphylococcus aureus* whereas *Fusarium culmorum* and *Fusarium sambucinum* sometimes showed activity against it. None of their Fusaria showed activity against *Bacterium coli.* Lacey (1950) suggested that antibiotic compounds were often produced by Fusaria but that they were rapidly destroyed by many strains of this fungus and, therefore, not detected; it is not known whether the strains studied here behaved in this way.

General Discussion

The adaptive powers of fungi are considerable due to their ability to form induced enzymes and to mutate and sometimes also to heterokaryosis (Jinks 1952–3; Stanier 1953). These adaptive powers must be of considerable advantage to the fungi in natural habitats with their variety of organic materials, but they can make laboratory examinations difficult due to strain instability. During the present investigation, changes in the growth rates and pigmentation of strains have sometimes been noted and it is possible that less obvious changes may also have taken place. The results should, nevertheless, be of value in determining at least the potential physiological properties of the fungi.

A summary of the crop yields of the fungi on different groups of substrates is given in table 16.

Comparison Between Different Strains

Only slight differences were found between the strains of Sterile Mycelium or *Rhizoctonia solani* from different grassland soils or between the strains of *Cylindrocarpon radicicola* isolated from soils under tussock grassland, forest, or pastures. It is apparent that the organic substrates provided by these different vegetational covers have not resulted in the establishment of nutritionally distinct strains of fungi.

Although the infrequent strains *Rhizoctonia* sp. 5P and *Fusarium oxysporum* 17T grew less vigorously than the predominant strains of these species on most substrates, the comparatively infrequent strains of *Cylindrocarpon radicicola,* 10P and 11P, from pasture soils grew at least as efficiently as the more abundant strains from forest or tussock-grassland soils.

If a general conclusion is permissible with relatively few strains, it would appear that there were no marked qualitative differences between strains which were predominant in a particular soil and those strains of the same species which occurred less abundantly in a different soil.

Comparison Between Different Species

All species of fungi grew well at 20°C but *Rhizoctonia solani* and the Fusaria grew best at higher temperatures. Growth of the non-sporing fungi was poor on media of low pH but *Fusarium oxysporum* and, to a less extent, *Cylindrocarpon radicicola* grew well in moderately acid media.

TABLE 16—Summary of Abilities to Utilise Substrates

Strain		Mean Yields, With Ranges in Parentheses, of Dry Mycelium, mg/100 mg Substrate Supplied. Substrate:			Mean Yields, With Ranges in Parentheses, of Dry Mycelium, mg/100 mg Glucose Supplied:		
					Nitrogen Substrate:		
		Carbohydrates and Alcohols	Aliphatic Acids	Aromatic Compounds	Pyrimidines	Purines and Uric Acid	Proteins and Nucleic Acids
Sterile Mycelium	1T	*25* (<1–44)	4 (0–8)	+	*0* (0–0)	*13* (1–27)	*34* (24–53)
	2P	27 (4–57)	6 (2–10)	+	*0* (0–0)	*20* (10–26)	*26* (18–32)
Rhizoctonia sp.	3T	30 (<1–57)	(–)	(–)	(–)	(–)	(–)
	4T	*35* (2–52)	*3* (0–10)	+	*7* (0–20)	*40* (25–52)	*58* (33–65)
	5P	*22* (3–42)	*3* (0–12)	+	*2* (0–5)	*11* (1–18)	*20* (9–30)
Rhizoctonia solani	6T	*29* (<1–53)	*8* (0–14)	+	*0* (0–0)	*24* (11–33)	*47* (19–65)
	7P	*31* (2–54)	*11* (2–20)	+	*0* (0–0)	*26* (3–41)	*49* (39–59)
Cylindrocarpon radicicola	8T	*29* (10–36)	*19* (14–26)	++	*7* (0–22)	*27* (22–29)	*33* (24–36)
	9F	*28* (13–36)	*13* (8–22)	++	*12* (0–37)	*36* (33–37)	*36* (23–41)
	10P	*29* (5–38)	*16* (10–22)	++	*10* (0–29)	*30* (29–31)	*36* (27–42)
	11P	*32* (12–42)	*19* (14–20)	++	*11* (0–33)	*37* (35–39)	*38* (33–41)
Fusarium culmorum	12P	*31* (5–40)	*14* (0–26)	++	*4* (0–11)	*27* (19–32)	*30* (13–40)
Fusarium sambucinum var. *coeruleum*	13T	*24* (5–32)	*18* (10–34)	++	*1* (0–4)	*27* (24–28)	*28* (17–35)
Fusarium oxysporum	14P	*31* (10–38)	*25* (20–30)	++	*0* (0–0)	*28* (21–32)	*32* (23–38)
	15P	*32* (15–37)	*26* (16–32)	++	*0* (0–0)	*29* (19–32)	*34* (20–43)
	16P	*31* (5–38)	*26* (18–32)	++	*0* (0–0)	*32* (26–34)	*34* (20–42)
	17T	*22* (6–31)	*16* (14–20)	++	*0* (0–0)	*13* (6–16)	*22* (14–27)

(–) Signifies no determination made;
+ signifies slight utilisation of the aromatic compounds:
++ signifies good utilisation of the aromatic compounds.

In most of the nutritional experiments, the members of the non-sporing fungi showed a marked resemblance to each other and in their poor growth with ribose and mannitol, aliphatic acids, aromatic compounds and solidified egg white, they could be distinguished from *Cylindrocarpon* and the Fusaria. The non-sporing fungi generally grew more slowly and used less glucose than *Cylindrocarpon* and the Fusaria but the Rhizoctoniae, in particular, assimilated this sugar very efficiently. Little fat was synthesised by the non-sporing fungi but large amounts were found in the mycelia of the sporing fungi.

The *Rhizoctonia* sp. and *Rhizoctonia solani* generally utilised the same substrates and could not easily be distinguished from each other by their physiological properties. The two strains of Sterile Mycelium, however, differed from most of the Rhizoctoniae in their requirements for thiamin and in their poor growth on sucrose media.

All strains of *Cylindrocarpon radicicola* could obtain their nitrogen from uracil and thus differed from the other fungi. Generally, however, *Cylindrocarpon* could not be distinguished from the Fusaria on the basis of their physiological properties.

Ecological Considerations

As the strains of soil fungi studied here were obtained from predominant species in New Zealand grassland soils, it would be expected that the determination of some of their physiological properties would help to explain their success as saprophytes in the soil.

The fungi could all grow well at temperatures of 16°–24°C. Under summer conditions and high soil temperatures, however, the Fusaria should be favoured as a temperature of about 28°C was optimal for their growth. It is thus of interest to note that *Fusarium oxysporum* has been found by Thornton (in press) to be predominant, in summer, in some introduced pasture soils.

The pH values of most of the grassland soils would be unlikely to influence the distribution of these fungi as they could grow in media over a wide pH range. It is, however, noteworthy that *Fusarium oxysporum* could grow under strongly acid conditions.

Garrett (1956) has listed four characteristics which favour high saprophytic ability of soil fungi, *viz.* (1) high growth rate and rapid germinability of spores; (2) good enzyme-producing equipment; (3) production of antibiotic toxins; (4) tolerance of antibiotics produced by other microorganisms. The properties of the fungi examined in the present work have, therefore, been considered under these headings.

(1) As these predominant fungi were isolated from the soil as actively growing hyphae, they must have been capable of spreading through the soil more extensively than most of the other saprophytic soil fungi. In the laboratory, also, most of the strains, except *Cylindrocarpon radicicola,* multiplied by means of diffuse, spreading hyphae rather than as dense, compact colonies. This type of growth, however, is characteristic of other fungi found in soil, *e.g.* Mucorales, and cannot be the sole reason for the isolation.

by the screened immersion plate technique, of the predominant species studied here. This spreading growth, nevertheless, must be of considerable value in the soil, especially to the non-sporing fungi.

Rapid linear growth of mycelium was considered by Hawker (1957) to be of considerable value for survival of many fungi in soil. The growth rates of the species of fungi studied here differed considerably but the strains of Sterile Mycelium and the *Rhizoctonia* sp. usually grew more slowly than strains of the other fungi. As the *Rhizoctonia* sp. occurred abundantly in tussock-grassland soils, it would appear that there is no simple correlation between the growth rates of these fungi and their distribution in nature.

(2) Judged by their growth with many different carbon and nitrogen substrates, all strains of these fungi possessed the ability to synthesise many different enzymes. There was, however, a marked difference between the groups of non-sporing and sporing fungi. The non-sporing fungi appeared to be much more discriminating between different carbon sources. This was particularly striking in the utilisation of carbohydrates and alcohols and well reflected in the coefficients of variation (table 6) which differed greatly for the two groups, being 47–79% and 21–35% for the non-sporing and sporing fungi respectively. This was also true to a considerable degree in the utilisation of aliphatic acids where only three overlapping coefficients of variation were found between the two groups (table 9). This division between the two groups applied, also, to the lignin degradation products (table 10). In spite of their rather restricted ability to utilise carbon sources, these non-sporing fungi are, however, commonly found in tussock-grassland soils and are also found in some introduced pasture soils in New Zealand (Thornton in press).

In grassland soils with their large quantities of plant roots, the actively growing fungi would be expected to be capable of utilising dead root materials. All the fungi studied here can grow on simple carbohydrate substrates but possess, in addition, a marked ability to grow on complex carbohydrates such as pectin, hemicellulose and cellulose. *Cylindrocarpon* and the Fusaria are also capable of utilising lignin degradation products. These fungi thus possess the necessary enzyme systems to make use of complex root materials and their predominance in a particular environment is not likely to be limited mainly by nutritional factors.

All the fungi incorporated a considerable proportion of the carbon of different carbohydrates into cell material. Large amounts of non-volatile metabolic products extractable by organic solvents were not excreted into the culture fluids after growth on glucose media. *Cylindrocarpon* and the Fusaria could also assimilate a considerable amount of the carbon of aliphatic acids. Under natural conditions with a limited supply of available food, this ability of the fungi to utilise many different carbon substrates efficiently for cell synthesis would be of considerable value.

These fungi could use nitrate and ammonium compounds and it is probable that they could use many different amino acids for their nitrogen requirements. The pyrimidines were generally not readily attacked but the ability of the fungi to use complex sources of nitrogen such as purines or proteins could be of considerable advantage in the soil.

(3) Although weak antifungal properties were shown by some strains of Sterile Mycelium, *Cylindrocarpon radicicola* and *Fusarium oxysporum,* none of the fungi produced antibacterial compounds. It is possible that their synthetic ability would be greater in their natural environments, but it seems reasonable to conclude that the production of antibiotic compounds would not be an important factor for the survival of these fungi in the soil.

(4) The fungi have not been examined as yet for their ability to tolerate antibiotic compounds produced by other micro-organisms. Such a study could reveal properties of much ecological significance as Brown and Wood (1953) and Garrett (1956) stated that this ability was necessary for an organism to remain as a coloniser of the soil. As a result of her work on the physiology of soil and leaf yeasts, di Menna (1959) believes that inhibitory secretions from other living organisms were mainly responsible for the distribution patterns of yeasts in nature.

In 1953, Brown and Wood claimed that it was not possible to state with any degree of precision what particular adaptations enabled a soil fungus to be predominant in some situations and either absent or subordinate in others. Park (1955), also, believed that it might be arbitrary to attempt to ascribe successful habitation in the soil to specific physiological characters. Garrett (1956) considered that even small differences in saprophytic ability, acting over a wide span of space and time in the natural environment, could be of decisive importance in saprophytic competition. It is thus not possible to state which characteristics of each species of fungus studied here were most important for the survival or predominance of the species in different soils. It can be concluded, however, that the general growth characteristics and marked nutritional abilities of these fungi would be of considerable importance in determining their success as saprophytes in the soil.

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PARASITES OF THE EUROPEAN RABBIT, *ORYCTOLAGUS CUNICULUS* (L.), ON SOME SUBANTARCTIC ISLANDS

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Summary

The following parasites were recovered from the alimentary tracts and fur of rabbits collected at Kerguelen Island (1 rabbit), Macquarie Island (9 rabbits), and Auckland Islands (123 rabbits from Rose Island and 146 from Enderby): 6 species of coccidia (*Eimeria stiedae* and 5 other species tentatively identified from unsporulated oocysts as *E. perforans, E. Piriformis, E. flavescens, E. irresidua* and *E. magna*), 3 nematodes (*Passalurus ambiguus, Trichostrongylus retortaeformis* and *Graphidium strigosum*), 3 mites (*Listrophorus gibbus, Cheletiella parasitivorax* and *Eutrombicula hirsti*) and a louse (*Haemodipsus ventricosus*). Only one specimen of *E. hirsti* was found (Macquarie I.). The absence of *G. strigosum* from Macquarie and of *P. ambiguus* from Rose and Enderby Islands is considered real, but the failure to find certain other species on some of the islands may be explained by the small number of rabbits examined. Rabbits on Rose and Enderby Islands had unusually heavy infestations of *T. retortaeformis* and of *G. strigosum* respectively. The parasitic fauna of rabbits on the subantarctic islands resembles that in Australia and New Zealand, but lacks several parasites characteristic of rabbits in Europe. Parasites with direct life-histories, brief non-parasitic stages and high rates of infestation have been the most successful in accompanying rabbits to new countries.

INTRODUCTION

Lists of the parasites found in introduced animals in various parts of the world are useful in showing the range of conditions that can be tolerated by different parasites, and in suggesting some of the reasons why certain species are more successful than others in accompanying their hosts to new countries. The rabbit is of particular interest in this respect because it is infested by a wide range of parasites and has been introduced to many different parts of the world (Thompson and Worden, 1956). The only information on the parasites of rabbits from subantarctic islands is provided by Johnston and Mawson (1945) who searched for nematodes in two rabbits, one from Kerguelen and one from Macquarie Island. The Kerguelen rabbit was infested with the pin worm *Passalurus ambiguus* but the Macquarie one contained no nematodes. The present paper provides a list of the parasites found in rabbits collected at Rose and Enderby Islands (Auckland Island Group) and at Kerguelen and Macquarie Islands; it also suggests some of the factors that have prevented the establishment of certain parasites on these islands.

The location of the islands is shown in Fig. 1, and the climate is described by Jeannel (1941) for Kerguelen, Barnett (1950) for the Aucklands and Taylor (1955) for Macquarie. The main features of the climate are the strong winds, high humidity, and the small seasonal variations in temperature.

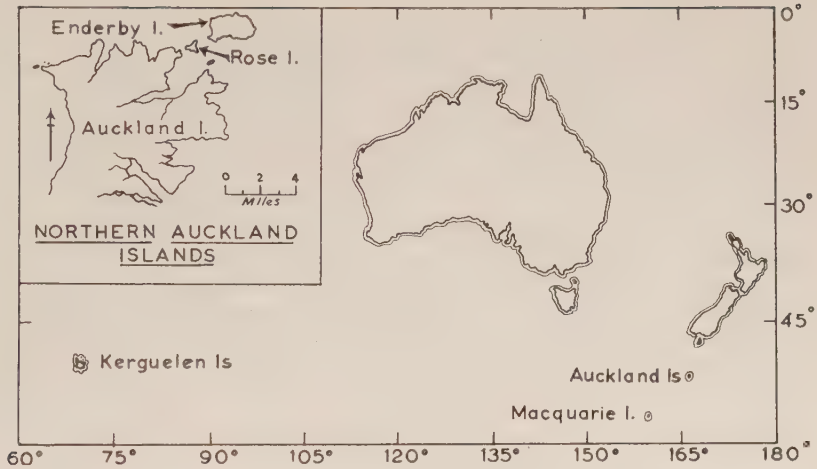

FIG. 1—Location of islands from which rabbits were collected.

Mean annual temperatures at sea level range from about 38°F at Kerguelen to about 46°F at Port Ross at the north end of Auckland Island. At Macquarie Island (mean temperature 40°F) the difference between the means of the hottest and the coldest months is only 6°F.

Rabbits were introduced to Rose Island about 1851 (Enderby, 1883), to Enderby Island in 1865 (Norman and Musgrave, 1866), to Kerguelen in 1874 (Paulian, 1953) and to Macquarie Island about 1880 (Taylor, 1955). An earlier introduction to Enderby Island in 1840 (Ross, 1847) was apparently unsuccessful because there were no rabbits there when Norman and Musgrave arrived in 1865. The rabbits were introduced to the islands as an emergency food for sealers and shipwrecked mariners, and the stocks for liberation probably came from England, either directly or by way of Australia.

MATERIAL AND METHODS

The Kerguelen and Macquarie Island material was collected by the Australian National Antarctic Research Expedition in February and March 1953 and consisted of livers and alimentary tracts from 10 rabbits (one from Kerguelen) and samples of skin and fur from 11 rabbits (two from Kerguelen). The skin and fur, containing ectoparasites, were preserved in alcohol, the remainder in formalin. All the rabbits were full-grown except for two that were half-grown (one from each island). The Macquarie Island rabbits were collected in equal numbers at three localities (Finch Creek, Prion Lake, and Island Lake) towards the northern end of the island.

Samples of 54 rabbits from Enderby Island and 21 from Rose Island were shot by Mr R. H. Taylor and the writer in March 1954; about half the rabbits from each island were young ones with paunched weights (Watson and Williams, 1955) of less than 1,000 g (i.e., under six months old). In the field, the animals were cut open and the livers examined for lesions and

the body cavity for cestode cysts. The livers and alimentary tracts were preserved in formalin for later examination in the laboratory. Samples of skin and fur from two rabbits from each island were preserved in alcohol.

A further 102 full-grown rabbits were shot on Rose Island and 92 on Enderby Island, in November 1954 by Dr K. Wodzicki and Mr R. H. Taylor (Animal Ecology Section, D.S.I.R.). The animals were examined as before, except that livers, caeca, and large intestines were discarded and no fur was preserved. Leg bones and lumbar vertebrae were retained and later used to assess the ages of the full-grown rabbits (Taylor, 1959). Laboratory procedures were the same as in earlier work (Bull, 1953).

Specimens of the parasites have been retained in the collection of Animal Ecology Section, Wellington, and a collection of parasites from Kerguelen and Macquarie Islands has been deposited in the National Museum of Victoria, Melbourne.

RESULTS

PROTOZOA

The Genus *Eimeria*

Of the 12 species of *Eimeria* recorded from the European rabbit by Becker (1956), 11 are found in the intestines and one (*E. stiedae*) in the liver. Representative samples of sporulated oocysts are required for the reliable identification of some of the intestinal species, but the present material was preserved before sporulation could occur. The results are presented therefore in two parts, the first dealing with oocysts found in the gall bladder (*E. stiedae*) and the second with oocysts recovered from rectal faeces (other species of *Eimeria*).

Eimeria stiedae (Lindemann, 1865)

Oocysts of *E. stiedae* were found in the gall bladders of rabbits from Macquarie, Rose, and Enderby Islands; the one set of viscera collected at Kerguelen was from a full-grown rabbit and contained no *E. stiedae*. Only two of the nine rabbits from Macquarie Island were infected, both contained small numbers of oocysts most of which were small and vacuolated as was to be expected in rabbits more than half-grown (Bull, 1958).

TABLE 1—Infections of *E. stiedae* in Young Rabbits

(300–800 g paunched weight) from Rose and Enderby Islands, March 1954

Method of Examination	Island	Rabbits		
		No. Examined	No. Infected	No. Severely Infected*
Macroscopic (Liver Lesions)	Rose	9	0	0
	Enderby	17	16	11
Microscopic (Oocysts in bile)	Rose	9	5	2
	Enderby	9	6	5

*Severely infected means more than half the liver affected by lesions, or an average of more than 5 oocysts per microscope field (low power).

Infections of *E. stiedae* were more severe on Enderby Island than on Rose Island, judging by the frequency of liver lesions and of oocysts in young rabbits (300–800 g paunched weight) collected there in March 1954, (table 1). The higher frequency of lesions on Enderby Island is significant ($P < 0.01$) but that of oocysts is not; fewer rabbits were examined for oocysts than for lesions. The mean lengths of oocysts from the gall bladders of rabbits from both Rose and Enderby Islands were about 2μ less than those recorded in the published descriptions of *E. stiedae* (table 3), but no significance is attached to this because the oocysts were from such a small number of rabbits; in certain other species of *Eimeria* the size of the oocysts increases during the patent period (Cheissin, 1947a; Becker *et al.*, 1956) while in some species (Cheissin, 1947a), but not others (Duncan, 1959), the size of the oocysts diminishes with increase in the number of oocysts that gave rise to the infection. The Rose Island samples in table 3 are of oocysts from a half-grown rabbit that contained both normal and vacuolated oocysts in its gall bladder and was probably recovering from the infection; the vacuolated oocysts were much smaller than the normal ones.

Though probably not caused by *E. stiedae*, it is convenient here to describe lesions found in the livers of full-grown rabbits collected on Rose and Enderby Islands in November 1954. These lesions, which were harder, whiter and more localised than those characteristic of *E. stiedae*, were found in 18% of 102 rabbits from Rose Island and in 21% of 92 rabbits from Enderby. The lesions were significantly more frequent in females than in males and in old females than in young ones (table 2). With *E. stiedae*, old rabbits normally have fewer lesions than do younger ones (Bull, 1958), but the reverse was found with this other type of lesion. None of the material was preserved and the cause of the condition remains unknown.

TABLE 2—Frequency of Liver Lesions in Full-grown Rabbits Collected on Rose and Enderby Islands in November 1954

Age of Rabbits	Males			Females		
	No. of Rabbits	No. With Lesions	% With Lesions	No. of Rabbits	No. With Lesions	% With Lesions
6–10 months	10	0	0%	24	1	4%†
10–33 ,,	36	4	11%	51	12	24%
> 33 ,,	35	5	14%*	38	15	40%*†
Total	81	9	11%	113	28	25%

*$P = 0.02$–0.05 for females > 33 months old, compared with males of this age.
†$P < 0.01$ for females > 33 months old, compared with females < 10 months old.

Other Species of *Eimeria*

Oocysts from the rectal faeces of rabbits from Kerguelen, Macquarie, Rose, and Enderby Islands varied greatly in size and shape (table 3) and several species are evidently present on each island. Five types were recognised in rabbits that contained no *E. stiedae* in the gall bladder.

TABLE 3—Measurements of Oocysts of *Eimeria* spp.

Species of *Eimeria* or Type of Oocyst	Locality of Collection	No. Oocysts Measured	No. Rabbits Providing Sample	Length (μ)			Width (μ)			Shape Index (Length/Width)
				Min.	Mean	Max.	Min.	Mean	Max.	
E. stiedae	Macquarie Island	13*	2	28	32·1	36	17	18·5	21	1·7
E. stiedae	Rose Island	50	1	29	34·9	40	16	20·7	22	1·7
E. stiedae	Rose Island	25*	1	26	30·9	33	15	16·3	18	1·9
E. stiedae	Enderby Island	256	4	29	34·2	39	18	20·3	24	1·7
E. stiedae	U.S.A. (Carvalho, 1943)	–	–	28	37·0	42	16	20·5	25	1·8
Type 1	Kerguelen Island	10	1	15	19·9	25	11	13·3	16	1·5
Type 1	Macquarie Island	37	6	16	20·8	28	11	14·0	18	1·5
Type 1	Rose Island	71	2	18	24·0	32	13	16·3	21	1·5
Type 1	Enderby Island	80	17	14	21·4	32	11	14·5	18	1·5
E. perforans	U.S.A. (Carvalho, 1943)	–	–	15	21·5	30	11	15·5	20	1·4
Type 2	Kerguelen Island	2	1	30	32·0	34	18	19·0	20	1·7
Type 2	Macquarie Island	51	9	26	30·5	36	17	19·1	22	1·6
Type 2	Rose Island	83	4	26	30·7	34	16	19·0	22	1·6
Type 2	Enderby Island	28	15	26	31·5	36	17	19·5	22	1·6
E. piriformis	Europe (Kotlán and Pellérdy, 1949)	–	–	26	29·0	32	17	18·0	21	1·6
Type 3	Kerguelen Island	7**	1	31	32·1	33	21	22·1	23	1·5
Types 3 and 4	Macquarie Island	71**	9	25	32·9	40	17	20·9	24	1·6
Types 3 and 4	Rose Island	43**	3	28	32·1	40	19	21·5	27	1·5
Types 3 and 4	Enderby Island	74**	14	27	33·6	45	18	21·8	26	1·5
E. flavescens	Europe (Marotel and Guilhon, 1941)	–	–	28	–	32	20	–	22	1·4
E. irresidua	U.S.A. (Kessel and Jankiewicz, 1931)	–	–	31	38·3	43	22	25·6	27	1·5
Type 5	Rose Island	1	1	–	35·0	–	–	23·0	–	1·5
Type 5	Enderby Island	103**	7	25	32·5	40	15	20·6	26	1·6
E. magna	U.S.A. (Carvalho, 1943)	–	–	28	35·0	42	20	24·0	26	1·4
E. coecicola	Europe (Cheissin, 1947b)	–	–	25	34·3†	40	15	18·3†	21	1·9†
E. elongata	Europe (Marotel and Guilhon, 1941)	–	–	35	–	40	17	–	20	2·0
E. exigua	U.S.A. (Carvalho, 1943)	–	–	12	15·7	24	9	13·0	16	1·3
E. matsubayashii	Japan (Tsunoda, 1952)	–	–	22·4	24·8	28·9	15·8	18·2	21·8	1·4
E. media	U.S.A. (Kessel and Jankiewicz, 1931)	–	–	27	31·2	36	15	18·5	22	1·7
E. nana	Europe (Marotel and Guilhon, 1941)	–	–	15	–	20	12	–	14	1·4

* Oocysts vacuolated. ** Some from rabbits that contained no *E. stiedae* in the gall bladder. † Approximated from the range of means recorded by Cheissin (1947b).

Type 1 oocysts are small and oval and appear to have no micropyle; the curvatures of the two extremities are equal. In size and appearance the oocysts conform to the description of *E. perforans* (Leuckart, 1879), and it is likely that most, if not all, were of this species. Two other species (*E. exigua* and *E. nana,* table 3) have small oval oocysts without micropyles, and oocysts grouped in Type 1 may therefore include more than one species. The larger oocysts, however, can only be *E. perforans*. Type 1 oocysts were common in rabbits from each of the four islands.

Type 2 oocysts are larger (table 3) and have one end broadly rounded and the other almost pointed; the rather narrow micropyle is situated at the more pointed end. The oocysts are fairly uniform in shape and size; similar oocysts from rabbits collected in New Zealand had no extra residual body after sporulation (Bull, 1953). Type 2 oocysts are tentatively identified as *E. piriformis,* Kotlán and Pospesch, 1934. Light infections were found in most of the rabbits from each of the four islands.

Oocysts of Types 3 and 4 differed from those of Type 2 in having both extremities broadly rounded and in the micropyle being wide and conspicuous. The oocysts varied greatly in size, and probably at least two species were present. The smaller oocysts (Type 3) are tentatively identified as *E. flavescens* (Marotel and Guilhon, 1941) since they were of the right size and since similar oocysts from New Zealand had no extra residual body (Bull, 1953). Type 3 oocysts were common in rabbits from each of the four islands. Type 4 oocysts, defined as those 35μ to 40μ long, are too large for *E. flavescens* and are tentatively identified as *E. irresidua* (Kessel and Jankiewicz, 1931). They were found in at least one rabbit from each of the Macquarie, Rose, and Enderby Islands, but they were much less common than oocysts of types 1–3. There were no type 4 oocysts in the one rabbit from Kerguelen Island.

The fifth type of oocyst is characterised by the presence of raised thickenings of the wall round the micropyle, a feature which is peculiar to *E. magna* (Pérard, 1925). Type 5 oocysts were numerous in two very young rabbits from Enderby Island and a few were found in other young rabbits from Enderby and also from Rose Island. None was found in the rabbits from Kerguelen and Macquarie Islands. Possibly this species, like *E. stiedae,* is more common in young than in full-grown rabbits and, if so, the Macquarie and Kerguelen Island rabbits, being mostly full-grown, would be unlikely to have them.

The present observations on the distribution of *Eimeria* spp. may be summarised as follows:

Kerguelen I., at least three species (probably *E. perforans, E. piriformis,* and *E. flavescens*).

Macquarie I., at least five species, all the Kerguelen ones plus E. *stiedae* and one other species (probably *E. irresidua*).

Rose and Enderby Is., at least six species on each island, all the Macquarie ones plus one other species (probably *E. magna*).

Further collecting of young rabbits from Kerguelen and Macquarie Islands, and the study of sporulated oocysts, would doubtless result in several additions to the above list.

TREMATODA AND CESTODA

No trematodes or cestodes were found in any of the rabbits examined.

NEMATODA

Passalurus ambiguus (Rudolphi, 1819)

This species was found in a rabbit collected at Kerguelen in 1929–30 (Johnston and Mawson, 1945), but it was not present in the one Kerguelen rabbit examined in the present study. *P. ambiguus* was found in two of the nine rabbits from Macquarie Island; one rabbit contained 10 worms and the other about 1,250.

P. ambiguus was not found on either Rose or Enderby Islands and it is fairly certain that the parasite does not occur there. Caeca and large intestines from rabbits of various ages, collected in March 1954, were examined in the laboratory (21 sets from Rose Island and 30 from Enderby), but no *P. ambiguus* was found. A further eight full-grown rabbits examined in the field on Enderby Island at this time were also negative. Finally, Dr K. Wodzicki and Mr R. H. Taylor failed to see any *P. ambiguus* when dissecting about 100 rabbits from each of Rose and Enderby Islands in November 1954. Wodzicki and Taylor were not specifically looking for *P. ambiguus*, but if the parasite had been abundant, it could hardly have been overlooked since the viscera of some of the rabbits had been ruptured by shot.

P. ambiguus is thus present on Kerguelen and Macquarie Islands but apparently not on Rose or Enderby Islands.

Trichostrongylus retortaeformis (Zeder, 1800)

T. retortaeformis was found in rabbits from Macquarie, Rose, and Enderby Islands, but not in the one rabbit from Kerguelen. It is possible that the parasite is absent from Kerguelen since Johnston and Mawson (1945) did not record it in the rabbit they examined from there.

In November 1954, when fairly large samples of full-grown rabbits were obtained from Rose and Enderby, infestations were very much heavier on the former island, and this difference held for all three age groups and for both sexes (Table 4). The high levels of infestation in females from Rose (382 worms per host) and Enderby (283 worms per host) are in agreement with New Zealand data (Bull, 1959) which show that the level of *T. retortaeformis* infestation in female rabbits, but not in males, reaches a peak in November (264 worms per host). Some of the young rabbits (under 1,000 g paunched weight) collected at the Auckland Islands in March had unusually heavy infestations. One young rabbit from Enderby contained some 20,000 worms and two others from Rose Island each had about 17,000 worms. The highest infestation yet recorded from New Zealand, where over 2,000 rabbits have been examined for *T. retortaeformis*, is only about 11,000 worms.

TABLE 4—*T. retortaeformis* Infestations in Relation to Sex and Age of Host and to Locality and Date of Collection

Locality and Date Sample Collected	Sex of Rabbits	Age of Rabbits	Number of Rabbits Examined	Number of Worms per Rabbit	
				Mean*	Max.*
				10	10
Rose Is. March 1954	Males	<6 months	3	1,120	1,296
		>6 „	3	13	23
	Females	<6 „	6	1,132	1,755
		>6 „	5	193	417
Enderby Is. March 1954	Males	<6 months	16	397	1,920
		>6 „	16	117	633
	Females	<6 „	9	326	467
		>6 „	13	66	220
Rose Is. November 1954	Males	6–10 months	7	253	794
		10–33 „	16	134	354
		>33 „	17	205	499
	Females	6–10 months	16	435	1,136
		10–33 „	25	317	914
		>33 „	18	425	1,036
Enderby Is. November 1954	Males	6–10 months	2	118	198
		10–33 „	18	39	191
		>33 „	16	89	316
	Females	6–10 months	8	271	1,092
		10–33 „	25	243	722
		>33 „	16	353	1,144
Macquarie Is.	Both Sexes	8 full-grown 1 half-grown	9	7	32
Kerguelen Is.	Male	1 half-grown	1	0	0

*The figures indicate the number of worms counted, i.e., 1/10 of those present.

No firm conclusions can be drawn concerning levels of *T. retortaeformis* infestation on Macquarie Island, as only nine rabbits were collected there. It is interesting, however, that, unlike the Auckland Island rabbits, the Macquarie Island ones were very lightly infested; the heaviest infestation consisted of only 315 worms and three rabbits contained no worms other than a few infective larvae. Likewise, Johnston and Mawson (1945) found no *T. retortaeformis* in the one Macquarie Island rabbit examined by them.

Graphidium strigosum (Dujardin, 1845)

G. strigosum was present in all of 163 rabbits from Rose and Enderby Islands, but it was not found in any of the nine rabbits from Macquarie Island nor in the one rabbit from Kerguelen. Likewise, the parasite was not found by Johnston and Mawson (1945) who examined one rabbit from each of Kerguelen and Macquarie Islands. Though small, the Macquarie Island sample strongly suggests that *G. strigosum* is absent from that island. The nine rabbits were collected in three different localities, each 2 miles apart; and eight of the rabbits were full-grown, such animals being more subject than young ones to infestation with *G. strigosum* (Bull and Taylor, 1956). The parasite may also be absent from Kerguelen, but insufficient rabbits have been examined to establish this.

At the Auckland Islands, highest levels of infestation were found on Rose Island in March and on Enderby in November. The high level of infestation in the small sample of rabbits collected at Rose Island in March may be fortuitous, but large samples were obtained from each island in November, and at that time infestations were about twice as high on Enderby as on Rose Island (table 5).

TABLE 5—*G. strigosum* Infestations in Relation to Sex and Age of Host and to Locality and Date of Collection

Locality and Date Sample Collected	Sex of Rabbits	Age of Rabbits	Number of Rabbits Examined	Number of Worms per Rabbit	
				Mean*	Max.*
				÷ 10	10
Rose Is. March 1954	Both Sexes	<6 months	9	72	150
		>6 ,,	7	332	684
Enderby Is. March 1954	Both Sexes	<6 months	14	17	40
		>6 ,,	21	183	830
Rose Is. November 1954	Males	6–10 months	5	92	189
		10–33 ,,	9	155	438
		>33 ,,	9	192	320
	Females	6–10 months	9	305	724
		10–33 ,,	14	286	781
		>33 ,,	9	342	552
Enderby Is. November 1954	Males	6–10 months	1	137	137
		10–33 ,,	13	441	773
		>33 ,,	12	652	1,364
	Females	6–10 months	6	290	544
		10–33 ,,	14	676	2,466
		>33 ,,	11	581	1,060

*The figures indicate the number of worms counted, i.e. $^1/_{10}$ of those present.

Levels of *G. strigosum* infestation at the Auckland Islands in 1954 were very much higher than those usually found in New Zealand, Australia, and Wales. The mean number of worms per host in 112 full-grown rabbits collected at the Auckland Islands in November 1954 was 4,000, whereas the highest level of infestation found by the author in New Zealand was 550 worms per host in a sample of 98 full-grown rabbits collected near Gore in August 1957 from a rabbit population as heavy as, or heavier than, those occurring at the Auckland Islands. The studies of Mykytowycz (1956) in Australia and of Evans (1940a) in Wales suggest that levels of *G. strigosum* infestation in those countries are no higher than those found in New Zealand, i.e., less than 600 worms per host. Although the level of *G. strigosum* infestation is influenced to some extent by the age-structure and sex ratio of the host sample (table 5), these influences are far too small to account for the fact that infestations of *G. strigosum* are some seven times heavier at the Auckland Islands than at other places where the parasite has been studied in the field.

ARTHROPODA

Ectoparasites recovered from the samples of skin and fur are listed below. The amount of material available for examination varied from rabbit to rabbit and from island to island and does not permit any quantitative assessment of rates of infestation or of the sizes of infestations.

Parasitic Mites

Specimens of each kind of mite in the Macquarie Island material were submitted for identification to Dr H. S. Womersley of the South Australian Museum. Apart from the species listed below, there were single damaged specimens of two species of Laelaptidae and several *Tyrophagus castellanii* Hirst, a tyroglyphid mite which is common in stored products and organic material and which probably invaded the fur when the rabbits were dead (H. Womersley, *in litt.* 10/6/58).

Listrophorus gibbus (Pagenstecher, 1861)

This mite was common in every sample of fur from each of the four islands.

Cheletiella parasitivorax (Megnin, 1878)

C. parasitivorax was found in one fur sample from each of Macquarie and Kerguelen Islands. The failure to find it in the few samples from Rose and Enderby Islands does not necessarily mean it was absent from there.

Eutrombicula hirsti (Sambon, 1927)

Dr Womersley found one in the collection of mites from Macquarie Island.

Haemodipsus ventricosus (Denny, 1842)

A few were recovered from rabbits collected at each of Macquarie, Rose, and Enderby Islands; none was found in the two samples of fur from Kerguelen Island.

DISCUSSION

DIFFERENCES IN DISTRIBUTION

Table 6 lists the parasites found by the author and by Johnson and Mawson (1945) in rabbits from Kerguelen, Macquarie, Rose, and Enderby Islands; the mite *Eutrombicula hirsti* has been omitted because, since only one specimen was found, this species may not be a normal parasite of rabbits. The species listed all occur in rabbits in Europe (references are given in Madsen, 1938, and Bull, 1953), but this is to be expected since Europe provided the rabbits for liberation in other parts of the world, and on the subantarctic islands there are no native land mammals from which rabbits might acquire new parasites. In Europe, however, rabbits have several additional parasites (see Hirst, 1922; Imperial Bureau of Agricultural Parasitology, 1931; Southern, 1940; and Becker, 1956). Some of the more conspicuous of these are the liver fluke *Fasciola hepatica,* the tape worms *Cittotaenia denticulata* and *C. pectinata,* the larval stages of the cestodes *Taenia serialis* and *T. pisiformis,* and the rabbit flea *Spilopsyllus cuniculi.* Except for the liver fluke and the two larval cestodes, these parasites also failed to become established in Australia and New Zealand (Bull, 1953, Mykytowycz, 1956 and 1957). Thus Australian and New Zealand rabbits contain a somewhat impoverished parasitic fauna compared with rabbits in Europe. This impoverishment is accentuated in the subantarctic islands, which as a group lack some of the parasites found in rabbits in Australia and New Zealand. Finally, the parasitic fauna of any one island may lack species that occur on other subantarctic islands. For instance among the nematodes, *Graphidium strigosum* occurs on the Auckland Islands but not on Macquarie, and *vice versa* with *Passalurus ambiguus.*

The species found at one or other of the subantarctic islands (table 6) are all one-host parasites with brief non-parasitic stages. The mites and lice are parasitic throughout life; the non-parasitic stages (oocysts, eggs or larvae) of the coccidia and nematodes occur in the host's faeces, where, provided conditions are moderately moist and warm, they become infective within a few days. Such parasites could probably complete their life cycles even under the highly unnatural conditions that exist when rabbits are held in captivity or transported overseas.

Parasites that are common in rabbits in Europe, but absent from the subantarctic islands, include cestodes and a liver-fluke, which require more than one host, and the rabbit flea, the larvae of which live among nest material in rabbit burrows and have rather strict humidity requirements. At least on Rose and Enderby Islands there are no suitable molluscs to provide intermediate hosts for *Fasciola hepatica* (R. K. Dell, pers. comm.) nor dogs to serve as primary hosts for *Taenia serialis* and *T. pisiformis.* The absence of

adult tapeworms (*Cittotaenia* spp.) from the subantarctic islands as well as from New Zealand (Bull, 1953) and Australia (Mykytowycz, 1956) may be explained by their relatively short life (Evans, 1940b) and the lack of intermediate hosts (probably an oribatid mite, Morgan and Hawkins, 1949) during the period of the hosts' captivity. Similarly, the larvae of the rabbit flea would probably not survive on the floors of rabbit cages. The absence from the subantarctic islands of all the above parasites can be explained therefore by the absence of a necessary second host (cestodes and flukes) or by unsuitable conditions for larval survival (fleas).

TABLE 6—List of Rabbit Parasites Found on Each Island

Species	Kerguelen Island	Macquarie Island	Rose Island	Enderby Island
Coccidia				
Eimeria stiedae	–	†	†	†
Eimeria type 1 (*E. perforans?*)	†	†	†	†
Eimeria type 2 (*E. piriformis?*)	†	†	†	†
Eimeria type 3 (*E. flavescens?*)	†	†	†	†
Eimeria type 4 (*E. irresidua?*)	–	†	†	†
Eimeria type 5 (*E. magna?*)	–	–	†	†
Nematodes				
Passalurus ambiguus	†*	†	x	x
Trichostrongylus retortaeformis	–	†	†	†
Graphidium strigosum	–	x	†	†
Acarina				
Listrophorus gibbus	†	†	†	†
Cheletiella parasitivorax	†	†	–	–
Anoplura				
Haemodipsus ventricosus	–	†	†	†

† = confirmed present.
– = not found.
x = almost certainly absent.
* Recorded by Johnston and Mawson (1945).

An explanation for why parasites such as *Passalurus ambiguus* and *Graphidium strigosum* occur on rabbits on some islands but not on others may be found in the fact that, at least in Australia and New Zealand, these species usually have lower rates of infestation than does *Trichostrongylus retortaeformis* (Mykytowycz, 1956, Bull, 1953). The low rates of infestation are mainly because *P. ambiguus* is relatively rare in old rabbits and *G. strigosum* in young ones. Under these circumstances, small samples of rabbits of fairly uniform age might occasionally be free of one or other of the parasites. So far as is known, the rabbit populations on the several islands have each arisen from a single effective liberation of a relatively small number of rabbits; 12 to Enderby Island (Norman and Musgrave, 1866) and a few pairs to Kerguelen Island (Paulian, 1953). Even if the worms did reach the islands, the initial population of susceptible rabbits may have been too dispersed to permit the parasites' survival. Other explanations for the absence of

P. ambiguus and *G. strigosum* from certain islands seem untenable. Both parasites occur in Australia and New Zealand as well as on some of the subantarctic islands, so they are evidently able to tolerate the conditions normally experienced during the captivity and transport of the host, and there is no reason to think that abnormal conditions, such as an unusually long voyage, would sometimes favour *P. ambiguus* and at other times *G. strigosum*. The absence of *P. ambiguus* from the Auckland Islands can hardly be due to an unfavourable environment for the eggs since the climate is intermediate between that of Macquarie Island and Southern New Zealand and the parasite occurs in both these places. Similarly, it is unlikely that *G. strigosum* which reaches such high infestations on Rose and Enderby Islands with their cool temperatures and acid soils, would find lethal the slightly colder temperatures of Macquarie Island.

Evidence for the absence of certain parasites from some of the islands is stronger for nematodes than for other groups and for this reason the preceding paragraph deals exclusively with nematodes. Other parasites such as *Eimeria irresidua, E. magna, Cheletiella parasitivorax* and *Haemodipsus ventricosus*, are also characterised by relatively low rates of infestation in rabbits in New Zealand (Bull, 1953), but the failure to find these species on some of the subantarctic islands is probably because too few rabbits were examined. This view is supported by the fact that the lists of coccidia from Rose and Enderby Islands are longer than those from other islands that contributed much smaller samples of viscera; similarly Macquarie Island, which provided the largest number of fur samples, has the longest list of ectoparasites (table 6).

Identical parasitic faunas were recovered from the Rose and Enderby Island rabbits (table 6). Though the original stocks of rabbits differed in colour and date of liberation, each probably contained the same parasitic fauna. It is possible, however, that birds have transported parasites across the narrow strait separating the two islands since skuas (*Stercorarius skua lonnbergi*) frequently prey upon the young rabbits. Strong currents would prevent rabbits swimming the strait, and the unusual but uniform colour of the Enderby Island rabbits (Sorensen, 1951) demonstrates that no Rose Island rabbits have reached Enderby Island. The experimental introduction of the nematode *Passalurus ambiguus* and the flea *Spilopsyllus cuniculi* to rabbits on one of these two islands, besides testing the ability of the parasites to exist in this environment, might usefully check the significance of birds in distributing parasites.

Differences in Abundance

By comparison with levels of infestation commonly found in New Zealand, infestations of *T. retortaeformis* on Rose and Enderby Islands were high, and those of *G. strigosum* very high. This was to be expected because the high density of rabbits that has developed in the absence of human control would ensure that the ground was heavily contaminated with parasite eggs, and the damp climate would permit a high rate of survival for the eggs and larvae. It is surprising, therefore, that infestations of *T. retortaeformis* on Macquarie Island were apparently low despite a high density of rabbits and a damp climate. Other factors must therefore be involved.

Marked differences in levels of infestation occur even between Rose and Enderby Islands. For instance, infestations of *T. retortaeformis* were much higher on Rose than on Enderby, while with *G. strigosum*, on the other hand, infestations were much higher on Enderby (except in the small sample collected in March); infections of *E. stiedae* were also more severe on Enderby. The two islands are close together and rather similar with regard to climate and to the density and age-structure of the rabbit populations so these factors are unlikely to explain the differences in levels of nematode infestation. The fact that the two nematodes reached maximum levels of infestation on different islands might suggest that there is some competition between the two species, a heavy infestation of one species tending to check the establishment of the other. Statistical tests, however, provided no evidence to support such an hypothesis. Another possibility is that the different genetic constitutions of the two rabbit populations is reflected in differing resistance to parasitism, as occurs between different breeds of sheep (Stewart *et al.*, 1937), but it seems unlikely that any such resistance would be effective against *T. retortaeformis* and not against *G. strigosum*. It is more likely that each nematode shows fluctuations in abundance, a slow period of increase followed by self-cure in the rabbit, and that the two species are out of phase with each other and with their own species on the other island. The presence of a self-cure mechanism causing sudden changes in the level of *T. retortaeformis* infestation in wild rabbits has been reported from New Zealand (Bull, 1955 and 1959). On this hypothesis, the low infestations of *T. retortaeformis* on Macquarie Island would be ascribed to a recent onset of self-cure.

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PELAGIC AMPHIPODA OF THE N.Z.O.I. PACIFIC CRUISE, MARCH 1958

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Summary

Pelagic Amphipoda taken on the New Zealand Oceanographic Institute Pacific Cruise in March, 1958, are listed with some observations on their distribution and ecology. There is evidence of a discontinuity in faunal distribution occurring between 20° S and 30° S along latitude 176° E which corresponds to a hydrological difference. Many species listed do not occur south of this front and conversely some of the most important species in New Zealand waters do not appear in these collections. There is also evidence to support a provisional division of Hyperiid Amphipods in the New Zealand region into Tropical, Subtropical and Subantarctic Faunas.

INTRODUCTION

In the course of studies on the hydrology of the Southern Pacific Ocean around New Zealand, members of the N.Z. Oceanographic Institute carried out two Pacific Cruises on H.M.N.Z.F.A. *Tui* from Auckland to Fiji, Tonga, and surrounding waters in March and September of 1958. Although the cruises were primarily designed to obtain hydrological data, opportunity was taken to obtain biological and geological samples. Plankton tows with an N70 net were taken whenever possible. If time permitted, both vertical hauls from 0–250 metres and surface hauls for one hour were obtained; when this was not possible, only one or other of these samples was taken.

The species recorded in this paper were taken on the March Cruise.

Station positions and biological data are given in Fig. 1 and table 1. In the Amphipod section, conventional listing of distributional information has been omitted for reasons given elsewhere (Hurley, 1960). Literature has generally been restricted to key references and subsequent recent papers.

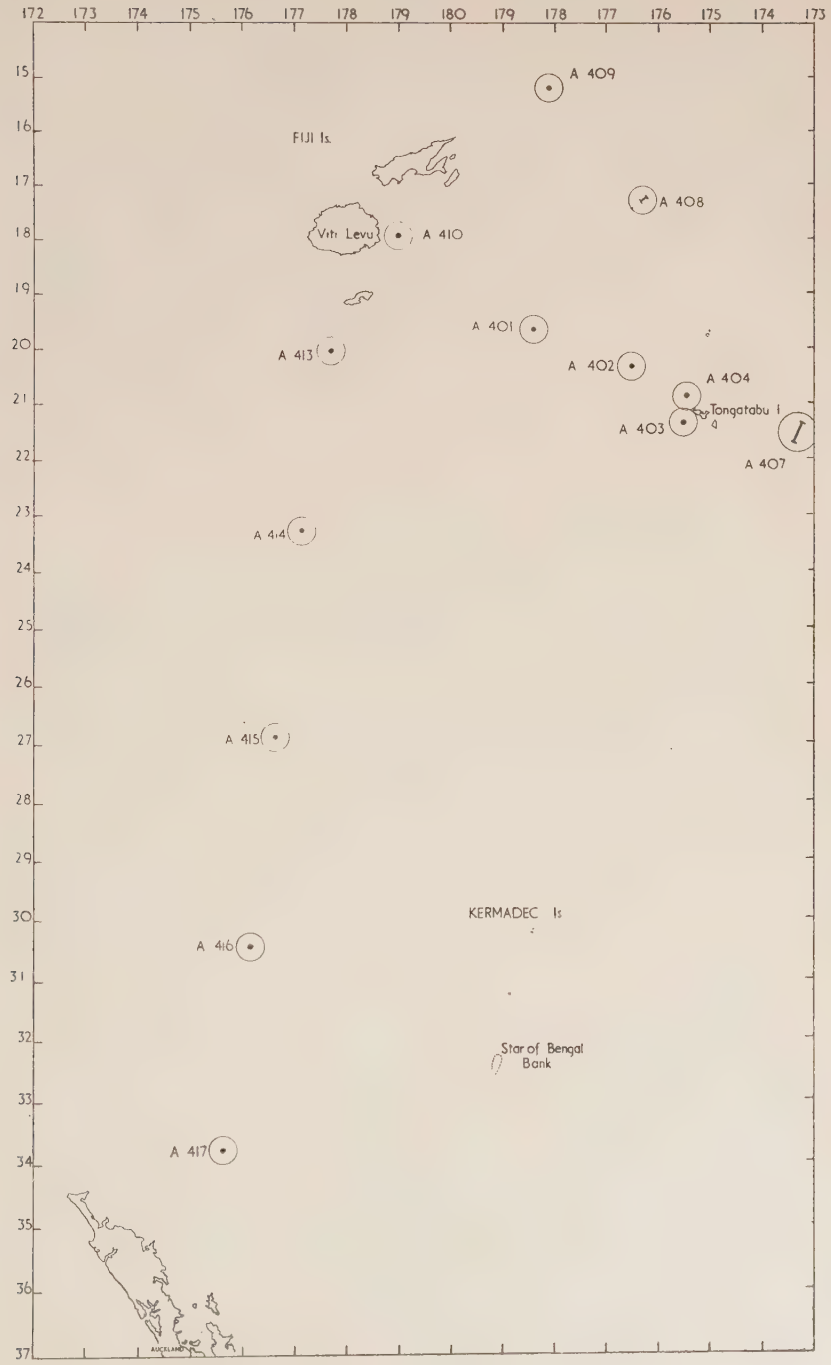

FIG. 1—Positions of stations at which pelagic amphipods were taken.

TABLE 1—Station Data*

(*Tui* Cruise, March 1958)

Station No.	Date	Latitude	Longitude	Time Start	Time Finish	Depth	Samples Taken
A. 401	16/3/58	19° 41′ S	178° 22′ W	1945	1800	640 fm	N. 70 Vertical 250–0 m.
A. 402	17/3/58	20° 19′ S	176° 31.8′ W	0800	0815	1,120 fm	N. 70 Vertical 250–m
		to 20° 21.2′ S	to 176° 29.3′ W	0830	0940		N. 70 Surface Net fouled with galley-gash
A. 403	17/3/58	21° 21′ S	175° 29.3′ W	2040	2055	595 fm	N. 70 Vertical 250–0 m
				2130	2255		N. 70 Surface
A. 404	18/3/58	20° 52′ S	175° 27.8′ W	0515	0615	1,070 fm	N. 70 Surface
				0700	0730		N. 70 Vertical 250–0 m
A. 407	19/3/58	21° 22′ S	173° 17.5′ W	1100	1200		N. 70 Surface
		to 21° 42.7′	to 173° 23′ W	1940	2040		N. 70 Surface (Net fouled from galley; plankton not kept)
	20/3/58			0130	0230		N. 70 Surface (Net fouled but plankton kept)
				1725	1755		N. 70 Vertical 250–0 m
A. 408	22/3/58	17° 15.7′ S	176° 16.8′ W	1300	1330		N. 70 Vertical 250–0 m
		to 17° 17′ S	to 176° 21′ W	1340	1440		N. 70 Surface
A. 409	23/3/58	15° 12′ S	178° 7′ W	1800	1820		N. 70 Vertical 250–0 m
				1830	1930		N. 70 Surface

A. 410	24/3/58	17° 57′ S	179° 00′ E	2130 2200	2155 2300	N. 70 Vertical 250–0 m N. 70 Surface
A. 413	28/3/58	20° 1′ S	177° 45′ E	0020	0040	N. 70 Vertical 250–0 m
A. 414	28/3/58	23° 16′ S	177° 10′ E	2000	2100	N. 70 Surface
A. 415	29/3/58	23° 54′ S	176° 38.5′ E	2050	2150	N. 70 Surface
A. 416	30/3/58	30° 27.2′ S	176° 7′ E	2040	2140	N. 70 Surface
A. 417	31/3/58	33° 48.8′ S	175° 39′ E	1835 1945	1935 2015	N. 70 Surface (Insufficient drift to stream net properly) N.70 10–0 m hand hauls. (= HH in text)

*Only biological data relevant to this paper given. Pelagic Amphipods were absent from tows taken at Stations A. 396 (Suva Harbour) and A. 412 (near Kandavu Island).

SYSTEMATICS

AMPHIPODA GAMMARIDEA

Family LYSIANASSIDAE

Parawaldeckia sp.

OCCURRENCE: A.403 (Vertical), 1 specimen; A.409 (Horizontal), 4 specimens.

REMARKS: Since this genus is in need of considerable revision, no attempt has been made to identify the species concerned. It is, however, surprising to find it in relatively deep water (595 fm) at some distance from the nearest land.

Family SYNOPIIDAE

Synopia ultramarina Dana, 1852

Synopia ultramarina, Shoemaker, 1945 : 195, text-fig. 8.
Synopia scheeleana Bovallius. Stebbing, 1888 : 799, pl. 52.

OCCURRENCE: A.401 (V), 1 male, 4½ mm; A.403 (H), 4 specimens, 2¼–4½ mm; A.407 (H), 2 females (one ovigerous), 3½ mm; A.410 (H), 1 specimen, 2½ mm; A.414 (H), 1 specimen, 3½ mm; A.415 (H), 9 specimens, 2¼–3½ mm.

REMARKS: These specimens agree closely with Stebbing's drawings of *S. scheeleana* in his Challenger report (Stebbing, 1888: pl. LII).

AMPHIPODA HYPERIIDEA

Family SCINIDAE

Scina crassicornis (Fabr.). Reid, 1955 : 12: Hurley, 1956 :8; Vinogradov, 1957 : 213.

OCCURRENCE: A.407 (H), 2 females, 7–9 mm; A.414 (H), 2 females, 5–9 mm, 1 intersex juv., 5 mm; A.415 (H), 1 female, 5 mm.

REMARKS: Vinogradov (1957) considers this a eurythermal species, ranging from the tropics to Antarctic waters of 0°C.

Scina damasii Pirlot, 1929 : 80–83, Fig. 7.

OCCURRENCE: A.409 (V), 1 female, 3 mm, ovigerous.

REMARKS: This species was described by Pirlot from "Armauer Hansen" stations off the Coast of Morocco, near the Canary Islands.

Family VIBILIIDAE

Vibilia antarctica Stebbing. Behning, 1925 : 486–8, Figs. 26–31; Barnard, 1932 : 263–4; Hurley, 1960 : 110, 111.

OCCURRENCE: A.403 (V), 1 juv., 3 mm; A.415 (H), 2 specimens, 3–4 mm.

Vibilia propinqua Stebbing. Behning, 1925 : 484–6, Figs. 23–25; Barnard 1932 : 263; Hurley, 1960 : 111.

OCCURRENCE: A.403 (H), 6 specimens, 4–8 mm; A.415 (H), 11 specimens, 4–7 mm.

Vibilia viatrix Bovallius, 1887. Barnard, 1932 : 262; Shoemaker, 1945 : 234; Reid, 1955 : 13; Hurley, 1960 : 111.

OCCURRENCE: A.417 (H H), 1 specimen, 6½ mm.

Vibilia armata Bovallius, 1887. Behning, 1925 : 491–4, Figs. 52–61: Barnard, 1932 : 264–5; Reid, 1955 : 13; Hurley, 1956 : 10.

OCCURRENCE: A.404 (V), 1 specimen, 2½ mm; A.410 (V), 1 specimen, 5 mm.

Vibilia chuni Behning & Woltereck, 1912: 8–9; Figs. 7–8. Behning, 1925: 496–8, Figs. 68–79.

OCCURRENCE: A.414 (H), 1 specimen, 5 mm.

Vibilia stebbingi Behning & Woltereck, 1912. Behning, 1925 : 482–4, Figs. 13–22; Hurley, 1955 : 125–9, Figs. 1–22; Hurley, 1960 : 111.

OCCURRENCE: A.417 (Hand Hauls), 1 specimen, 2 mm; A.417 (H), 1 specimen, 5 mm.

Family HYPERIIDAE

Hyperia bengalensis (Giles), 1887. Reid, 1955 : 16–17; Hurley, 1955 : 137; Hurley, 1956 : 14.

OCCURRENCE: A.403 (V), 1 male, 3 mm; A.403 (H), 2 males, 2½–3 mm, 1 female, 3 mm; A.404 (V), 2 males, 2½–3 mm, 1 female, ovigerous; A.404 (H), 2 males, 2½–4 mm; A.407 (H), 2 males, 3 mm; A.409 (H), 1 male, 3½ mm; A.413 (V), 1 specimen, 2½ mm; A.414 (H), 7 males, 3–3½ mm; A.415 (H), 149 specimens, 2–5 mm.

Hyperia luzoni Stebbing, 1888. Barnard, 1930 : 410; Pirlot, 1939 : 35.

OCCURRENCE: A.403 (H), 2 females, 1 juv. male, 2½–3 mm; A.407 (H), 3 males, 2½–3 mm; A.409 (H), 1 juv. male, 2½ mm; A.414 (H), 13 males, 2¾–3½ mm, 10 females, 1½–3 mm; A.416 (H), 1 juv. female, 2½ mm; A.417 (HH), 4 females, 2–3¾ mm.

Hyperia macrodactyla Stephensen, 1924 : 90–91, Fig. 35.

OCCURRENCE: A.410 (H), 1 male, 2 mm.

REMARKS: This specimen is like Stephensen's in the long peraeopod claws. The peduncle of Antenna 1 is of equal width throughout its length although an oblique view gives the narrowed appearance shown in Stephensen's figures. Gnathopod 2 is slightly shorter than Gn. 1, but longer than Peraeopod 1. The 5th segment of Gn. 2 has a short sharp process posterodistally.

Hyperia sp.

OCCURRENCE: A.410 (H), 1 unidentified fragment.

Hyperioides longipes Chevreux, 1900 : 143, pl. 17, Fig. 2. Chevreux & Fage, 1925 : 407–8, Fig. 405; Barnard, 1932 : 276; Shoemaker, 1945 : 238.

OCCURRENCE: A.407 (H), 2 males, 2½–3 mm; A.408 (V), 2 females, 3½–4 mm; A.409 (H), 1 male, 3 mm; A.414 (H), 2 males, 2½ mm.

Family PHRONIMIDAE

Phronima stebbingii Vosseler, 1901. Reid, 1955 : 20; Hurley, 1956 : 17.

OCCURRENCE: A.403 (V), 2 males, 3 mm, 2 females, 3–4 mm; A.404 (V), 1 male, 6 mm, 1 female, 4 mm; A.407 (V), 1 female, 5 mm; A.410 (H), 1 juv. male, 4 mm; A.415 (H), 6 males, 9 females, 1–5 mm.

Phronima colletti Bovallius, 1887. Reid, 1955 : 20; Hurley, 1956 : 17.

OCCURRENCE: A.403 (H), 1 juv., 2¾ mm; A.415 (H), 9 males, 4 females, 2 juv., 1–6 mm; A.417 (HH), 2 males, 3–7 mm.

Phronima sedentaria (Forsk.), 1775. Hurley, 1955 : 166, Figs. 188–218; Reid, 1955 : 21; Hurley, 1956 : 16; Hurley, 1960 : 113; Vinogradov, 1956 : 210.

OCCURRENCE: A.414 (H), 1 female, 5 males, 7 juv., 2–8 mm; A.415 (H), 1 female; A.416 (H), 6 males, 7–8 mm; A.417 (H), 2 males 8–12 mm; A.417 (HH), 5 females, 10–19 mm.

Phronimella elongata Claus, 1862. Chevreux & Fage, 1925 : 398–9, Fig. 400; Shoemaker, 1945 : 236–8; Reid, 1955 : 21.

OCCURRENCE: A.403 (V), 1 male, 8 mm, 1 female, 12 mm; A.415 (H), 2 males, 8 mm, 1 female, 13 mm.

Family PARAPHRONIMIDAE

Paraphronima crassipes Claus, 1879. Chevreux & Fage, 1925 : 390–1, Figs. 393–4; Reid, 1955 : 15, Fig. 3; Vinogradov, 1956 : 209; Hurley, 1960 : 113.

OCCURRENCE: A.415 (H), 1 male, 2½ mm, 1 female, 5 mm.

Family PHROSINIDAE

Primno macropa Guérin-Meneville, 1836. Hurley, 1955 : 172–4, Figs. 219–235; Reid, 1955 : 23; Vinogradov, 1956 : 209; Hurley, 1956 : 17; Hurley, 1960 : 113.

OCCURRENCE: A.402 (V), 3 juv., 1–2 mm; A.403 (V), 5 males, 3–6 mm; A.404 (V), 1 female, 4½ mm; A.407 (V), 1 juv., 2 mm; A.408 (V), 1 female, 2½ mm; A.415 (H), 1 female, 2½ mm.

Phrosina semilunata Risso, 1862. Reid, 1955 : 22; Hurley, 1956 : 18.

OCCURRENCE: A.409 (H), 1 female, 3½ mm; A.410 (H), 1 sp., 4 mm; A.414 (H), 1 male, 4½ mm.

Anchylomera blossevillei M. Edw., 1830. Reid, 1955 : 22; Hurley, 1956 : 18.

OCCURRENCE: A.402 (V), 95 juv., 1–5 mm; A.403 (V), 1 male, 5 mm, 2 females, 2–4 mm; A.403 (H); 6 males, 6–7 mm; A.407 (H), 1 female, 2 mm; A.409 (H), 678 females, 1–7 mm; 45 males, 1–6 mm, numerous ova and minute juveniles; A.409 (V), 7 males, 5–6 mm, 11 females, 3–6 mm; A.410 (H), 1 male; A.410 (V), 1 specimen, 2 mm; A.413 (V), 1 specimen; A.415 (H), 2 females, 2 mm; A.416 (H), 1 female, 3½ mm; A.417 (HH), 4 males, 4–6 mm, 56 females, 4–6 mm; A.417 (H), 2 males, 5½–6 mm, 10 females, 3–6 mm.

Family PRONOIDAE

Eupronoe minuta Claus, 1897. Reid, 1955 : 25; Hurley, 1956 : 19.

OCCURRENCE: A.403 (V), 1 male, 4 mm; A.403 (H), 3 males, 3½–5 mm; A.409 (H), 27 males, 4 females; A.410 (V), 1 specimen, 3 mm; A.410 (H), 5 males, 4 mm; A.413 (V), 1 male, 4 mm, 1 female, 2½ mm; A.414 (H), 21 specimens, 3–4½ mm; A.415 (H), 135 specimens, 3–5 mm.

Paralycaea gracilis Claus, 1879. Hurley, 1955 : 175–9, Figs. 236–51; Hurley, 1956 : 19.

OCCURRENCE: A.407 (H), 3 males, 2–5 mm; A.414 (H), 4 males, 2½ mm.

Parapronoe crustulum Claus, 1879. Shoemaker, 1945 : 246, figs. 40–41; Reid, 1955 : 24.

OCCURRENCE: A.410 (H), 1 male, 11 mm; A.413 (V), 1 male, 12 mm; A.414 (H), 1 male, 6 mm.

Sympronoe parva (Claus), 1879. Reid, 1955 : 23; Hurley, 1956 : 19.

OCCURRENCE: A.410 (H), 1 specimen, 5 mm; A.415 (H), 1 male, 3¼ mm.

Family LYCAEOPSIDAE

Lycaeopsis zamboangae (Stebbing), 1888. Hurley, 1956 : 20.

OCCURRENCE: A.403 (V), 2 males, 2¼–3½ mm; A.407 (H), 1 male, 4½ mm; A.408 (V), 1 juv., 1½ mm; A.414 (H), 1 juv. female, 3½ mm.

REMARKS: The adult of this species is easily recognised by the astonishingly long 4th-7th segments of peraeopod 3 (cf. Stebbing, 1888 : pl. CLXXX), but the juveniles, in which these segments are of relatively normal hyperiid length, are sometimes confusing. However, the shape of the head, the expanded 4th segment of Pr. 4, and the curiously incised inner ramus of uropod 3 are good indications as to their affinities.

Family LYCAEIDAE

Lycaea pulex Marion, 1874. Reid, 1955 : 25; Hurley, 1956 : 20.

OCCURRENCE: A.403 (H), 1 specimen, 3 mm; A.404 (H), 1 female, 2½ mm; A.410 (H), 1 male, 6 mm; 1 female, 3 mm; A.417 (HH), 1 female, 2½ mm.

Family BRACHYSCELIDAE

Brachyscelus crusculum Bate, 1861. Shoemaker, 1945 : 242; Reid, 1955 : 25.

OCCURRENCE: A.403 (H), 2 specimens, 2–2½ mm; A.414 (H), 2 juv., 1¾–2½ mm.

Family OXYCEPHALIDAE

Leptocotis tenuirostris (Claus), 1871. Shoemaker, 1945 : 253–4; Reid, 1955 : 28.

OCCURRENCE: A.414 (H), 1 male, 11 mm; A.415 (H), 2 females, 8–9 mm, 2 males, 11 mm.

Streetsia porcella (Claus), 1879. Shoemaker, 1945 : 255; Reid, 1955 : 29.

OCCURRENCE: A.410 (H), 1 male, 11 mm; A.413 (V), 1 male, 12 mm; A.414 (H), 1 juv. female, 3 mm.

Cranocephalus scleroticus (Streets), 1878. Shoemaker, 1945 : 251–3, Fig. 44; Reid, 1955 : 30, Figs. 7–8.

OCCURRENCE: A.409 (H), 1 female, 7 mm; A.415 (H), 1 ovig. female, 5½ mm.

Rhabdosoma whitei Bate, 1862. Shoemaker, 1945 : 255.

OCCURRENCE: A.404 (H), 1 specimen, 6 mm.

Family SCELIDAE

Parascelus typhoides Claus, 1879. Hurley, 1955 : 183; Hurley, 1956 : 21.

OCCURRENCE: A.410 (V), 1 male, 4½ mm; A.410 (H), 1 male, 4½ mm.

Parascelus similis Stephensen, 1925 : 209–211, Figs. 81–82.

OCCURRENCE: A.415 (H), 1 specimen.

REMARKS: This specimen seems to agree quite well with Stephensen's figures and to be specifically distinct from *Parascelus typhoides*, not an immature form of the latter.

Thyropus sphaeroma (Claus), 1879. Shoemaker, 1945 : 260; Reid, 1955 : 33.

OCCURRENCE: A.407 (H), 1 male, 5 mm; A.414 (H), 1 male, 4½ mm; 1 female, 3 mm; A.415 (H), 10 specimens, 5½ mm.

REMARKS: The head of this species is rather distinctly flattened and shield-like, almost spoon-shaped, not at all solid and globular.

Family PLATYSCELIDAE

Platyscelus serratulus (Stebbing), 1888. Reid, 1955 : 37; Hurley, 1956 : 21.

OCCURRENCE: A.404 (V), 1 specimen, 3 mm; A.409 (V), 1 male, 4½ mm; A.409 (H), 2 males, 3½–4 mm; A.410 (H), 6 specimens, 4 mm; A.413 (V), 1 specimen, 1¾ mm.

REMARKS: A rather prominent rostrum in this species helps to distinguish it from *Platyscelus ovoides.*

Platyscelus ovoides (Claus), 1879. Hurley, 1955 : 189–192, Figs. 272–290; Reid, 1955 : 36.

OCCURRENCE: A.414 (H), 21 specimens, 2½–4 mm.

Amphithyrus bispinosus Claus, 1879. Shoemaker, 1945 : 259; Reid, 1955 : 33.

OCCURRENCE: A.414 (H), 9 males, 2½–3 mm; A.415 (H), 2 males, 3½ mm.

Amphithyrus sculpturatus glaber Spandl, 1924. Pirlot, 1930 : 45; Pirlot, 1939 : 59.

OCCURRENCE: A.415 (H), 3 females, 1½–2½ mm.

Paratyphis maculatus Claus, 1879. Shoemaker, 1945 : 259–60; Reid, 1955 : 36.

OCCURRENCE: A.409 (H), 55 specimens, 2–2½ mm; A.413 (V), 1 male, 3½ mm; A.414 (H), 1 male, 3¼ mm.

DISTRIBUTION AND ECOLOGY

In all, 40 species of pelagic Amphipoda, mostly Hyperiidea, were taken on this cruise. (This excludes the unidentified *Parawaldeckia* specimens). Tabulation of species against latitude (table 2) shows immediately one striking break in distribution between the two southernmost stations, A.416 and A.417, and those to the north. Of the 40 species, only seven were taken at A.416 and A.417, but 20 were collected at A.415, the next northward station.

It is not difficult to find an explanation for this difference. Stations A.416 and A.415, in latitudes of 30° S and 27° S respectively, straddle the generally accepted region of a tropical "front" north of New Zealand. According to Deacon (1933) "the temperature of sub-tropical water in 30° W increases gradually towards the north until, in about 28° S (when the temperature at the surface is 23°C), the increase becomes rapid and another surface layer of water can be distinguished. This second layer lies above sub-tropical

TABLE 2—Tabulation of Species at *Tui* Stations
(H = Horizontal net tow; V = Vertical net tow to maximum depth of 250 metres)

	Haul	417	416	415	414	413	410	401	402	403	404	407	408	409
Lycaea pulex	H	1					2			1	1			
	V													
Hyperia luzoni	H	4	1		23					3		3		1
	V													
Phronima sedentaria	H	7	6	1	13									
	V													
Phronima colletti	H	2		15						1				
	V													
Anchylomera blossevillei	H	72	1	2			1			6		1		723
	V					1	1		95	3				18
Vibilia stebbingi	H	1												
	V													
Synopia ultramarina	H			9	1		1			4		2		
	V							1						
Cranocephalus scleroticus ..	H			1										1
	V													
Eupronoe minuta	H			135	21		1			3				31
	V					2	5			1				
Hyperia bengalensis	H			149	7					3	3	2		1
	V					1				1	2			
Amphithyrus bispinosus	H			2	9									
	V													
Parascelus similis	H			1										
	V													
Amphithyrus sculpturatus	H			3										
	V													
Sympronoe parva	H			1			1							
	V													
Leptocotis tenuirostris	H			4	1									
	V													
Phronima stebbingi	H			15			1							
	V									4	2	1		
Phronimella elongata	H			3										
	V									2				
Paraphronima crassipes	H			2										
	V													
Scina crassicornis	H			1	3							2		
	V													
Primno macropa	H			1										
	V								3	5	1	1	1	

TABLE 2—Tabulation of Species, etc.—*continued*

	Haul	417	416	415	414	413	410	401	402	403	404	407	408	409
Vibilia propinqua	H			11						6				
	V													
Vibilia antarctica	H			2										
	V									1				
Thyropus sphaeroma	H			10	2							1		
	V													
Brachyscelus crusculum	H				2					2				
	V													
Streetsia porcella	H				1		1							
	V					1								
Vibilia chuni	H				1									
	V													
Paralycaea gracilis	H				4							3		
	V													
Parapronoe crusculum	H				1		1							
	V					1								
Phrosina semilunata	H				1		1							1
	V													
Paratyphis maculatus	H				1									55
	V					1								
Lycaeopsis zamboangae	H				1							1		
	V									2			1	
Hyperioides longipes	H				2							2		1
	V												2	
Platyscelus ovoides	H				21									
	V													
Platyscelus serratulus	H						6							2
	V					1					1			1
Parascelus typhoides	H						1							
	V						1							
Vibilia armata	H													
	V						1				1			
Hyperia macrodactyla	H						1							
	V													
Rhabdosoma whitei	H										1			
	V													
Scina damasii	H													
	V													1
Vibilia viatrix	H													
	V													

water, and is separated from it by another discontinuity of temperature and salinity, forming so sharp a density gradient that vertical mixing between the two layers is almost entirely prevented. The surface layer has been called tropical water. It is almost depleted of its dissolved phosphate and nitrate; and they cannot be renewed, except perhaps very slowly, because the sharpness of the discontinuity makes vertical mixing across it almost impossible. The mean temperature in the discontinuity between the tropical water and the sub-tropical water is 23°C." North of New Zealand, Garner (1955) notes a surface temperature difference of 13°F in February, and 18°F in August between Suva, Fiji, and North Cape, New Zealand. "The gradient associated with this temperature difference was not smooth, temperature tending to change in a number of abrupt steps. The most pronounced of these, usually of some 4–6°F, tended to lie around latitude 30° S during January and around latitude 22° S during July. . . . Maximum sea temperature occurred in February and minimum in August or September at all latitudes."

Water temperatures on the *Tui* Cruise (Fig. 2) indicate a difference of approximately 2°–3°C between stations A.415 and A.416–417, corresponding to the expected position of the front. This temperature difference holds at depths of 100, 200, and 300 metres (as against a maximum net depth of 250 m), and is greater than that between any other two consecutive stations north of A.415, except at the 100 metre level where Stations A.415, A.414, and A.413 show a similar disparity with temperatures from more northerly or easterly stations (Fig. 2). Significantly, the surface temperature at A.416 was 23·3°C.

Salinities were also examined to a depth of 300 metres for possible correlation with hyperiid distribution but no obvious correlation was found. From the above information, however, it would seem that there is a faunal difference between pelagic amphipods north and south of the Tropical Front and this is primarily dependent on temperature although other, less obvious, factors may also be involved.

This faunal difference was, in fact, observed when the material was collected, although not with respect to amphipods. At Station A.415 and all stations to the north, the plankton showed a predominant blue colour, mainly contributed by copepods of both *Calanus*- and irridescent *Sapphirina*-like species. Station A.415 was particularly productive of plankton – this is reflected in the presence there of 20 species of hyperiids some in considerable numbers – and the blue copepod element was very noticeable. At Station A.416, the blue element was almost completely replaced by a distinct orange element, again due to brightly coloured copepods. In contrast to Station A.415, plankton at A.416 was noticeably sparse.

Four of the seven species of hyperiids taken at the two southern stations occur also at northern stations – these include so-called "cosmopolitan" species, e.g. *Phronima sedentaria*. It is likely, too, that other species taken at the northern stations will also be found south of the Tropical Front although missing on this occasion, e.g., *Hyperia bengalensis*, *Paralycaea gracilis*, *Primno macropa*, and *Platyscelus ovoides*. These species have previously been taken in southern New Zealand waters (Hurley, 1955; Bary, 1959).

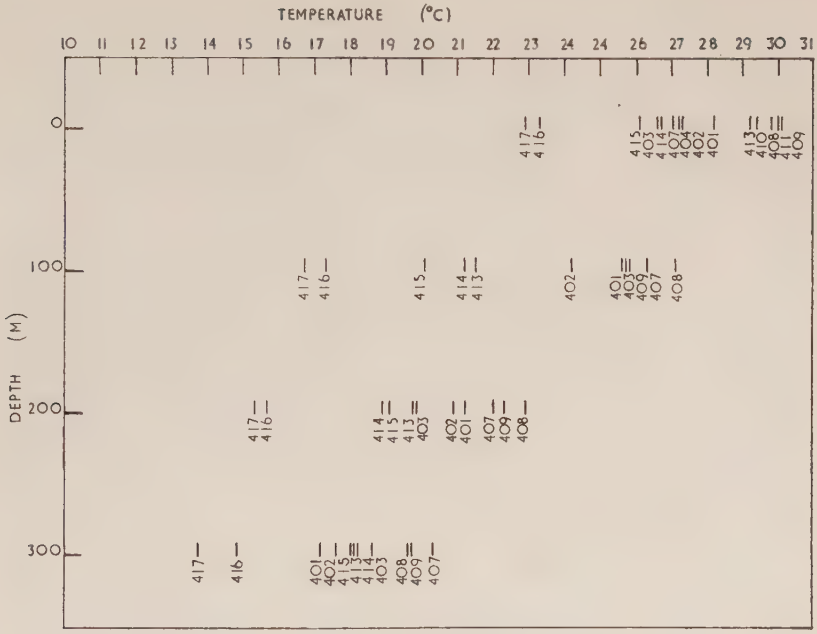

FIG. 2—Temperatures of seawater at the surface and at depths of 100, 200, and 300 metres at stations where pelagic amphipods were taken. Note the consistently lower temperatures at all depths of Stations A.416 and A.417.

There are other significant differences. Not one member of the bizarre Family Oxycephalidae was taken south of Station A.415, although specimens were taken at the next four stations northwards. Previous New Zealand records of this family are all from approximately 34° S in the vicinity of Three Kings Islands (Barnard, 1930). None was taken in southern New Zealand waters by the H.M.N.Z.S. *Lachlan* investigations (Hurley, 1955; Bary, 1959).

Even more strikingly, the two most important genera of Hyperiidea in New Zealand and Subantarctic waters, *Parathemisto* and *Cyllopus,* are entirely absent from the March 1958 Collections. On this evidence, it would seem that the Tropical Front represents a major boundary between warm and temperate hyperiid faunas in New Zealand waters.

Further confirmation, although based on limited material, is to be found in the distribution of hyperiid species taken by *Tui, Terra Nova* (Barnard, 1930), and *Lachlan* (Hurley, 1955; Bary, 1959) in the New Zealand region as far south as 55°S. Allowing for synonymy changes, these collections cover something like 74 species. Of these, 52 occur only in the Three Kings region or further north. Twelve species occur only south of Three Kings. Ten species occur in both regions. These are: *Phronima atlantica, Hyperioides longipes, Paraphronima crassipes, Parathemisto australis, Primno macropa, Phronima sedentaria, Vibilia armata, Hyperia bengalensis, Vibilia stebbingi, Paralycaea gracilis,* and *Platyscelus ovoides.*

There is little doubt that some of these species, e.g. *Phronima sedentaria* and *Primno macropa* have a wide temperature and salinity tolerance. (There is also the possibility that some have been wrongly identified.) However, it is also likely that some of the ten, e.g. *Parathemisto australis,* which was completely absent from *Tui* (March, 1958) Stations, are generally confined to one or other side of the temperature barrier provided by the Tropical Front but this fact has been masked by upwelling which is believed to occur in the hydrologically complex Three Kings region (for a discussion of this, see Garner, 1959) or by other factors.

One other point should be noticed. Apart from the *Terra Nova* Three Kings Stations, none of the *Lachlan* material referred to above or *Terra Nova* material was collected further north than Cook Strait (41° S), and almost all on the East Coast. This is south of the main boundary between subtropical and subantarctic water east of New Zealand. The Subtropical Convergence off the East Coast is found "usually between Cape Kidnappers and Palliser" (Garner, 1959a). It is therefore possible that the differences between the faunas north and south of the Tropical Front are not as great as suggested, because the lack of stations between 34° S and 41° S has resulted in the differences observed being actually the effect, not of one, but of two hydrological barriers.

Thus the striking fact that, of the 74 species considered, less than 15% are common to collections north and south of 34° S may be a comparison, not of tropical and subtropical faunas, but of tropical and subantarctic faunas. This should not, however, be allowed to conceal the point that significant differences exist between collections made on the March 1958 *Tui* Cruise north and south of the tropical front at 30° S. On the basis of this limited evidence, it is considered that a provisional distinction of the Amphipoda Hyperiida in the New Zealand region into Tropical, Subtropical, and Subantarctic Faunas is justified. The Subtropical Zone is bounded in New Zealand waters by the Tropical Front in the region of 30° S and the Subantarctic Convergence in the vicinity of Cook Strait on the East Coast of New Zealand and Foveaux Strait on the West Coast. A further boundary delimiting the Subantarctic Hyperiid Fauna to the South should be sought along the Antarctic Convergence between 60° and 65° S.

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NOTE ON BREADMAKING WITHOUT BULK FERMENTATION: THE EFFECT OF MIXING AND STAGE OF ADDITION OF POTASSIUM IODATE

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Traditional breadmaking processes include a fermentation stage of several hours duration in which the yeast and flour in the dough interact to give a maturing effect, the progress of which is marked by obvious changes in the dough and resulting bread. Loaves baked from under-fermented doughs are small, with a smooth crust, sharp corners and coarse crumb, and are described by bakers as "green". Over-fermented doughs produce small loaves with a rough, torn pale crust, rounded corners and ragged crumb; they are described as "over-mature". The optimum ("mature") loaf has a bold appearance, large volume, and fine silky crumb texture. The maturing effect is generally believed to be due to increased molecular cross-linking in the dough but the precise mechanisms involved in the change have not been elucidated. Oxidative treatment of flour and the inclusion of oxidising agents in dough have long been used to hasten or control maturity, especially in "short" doughs which may have a fermentation time of five hours or less. Generally the optimum amount of oxidising agent is of the order of 10–20 p.p.m. of flour, and a 50% variation in this amount will produce a definite fall in bread quality, especially with respect to texture and volume.

A recent development has been the introduction of continuous dough-mixers and the elimination of the batch-wise fermentation stage. Maturity in the dough is controlled in most of these new processes by the addition of potassium iodate, which is recognised as more rapid in its effect on dough than the usual additive, potassium bromate. Iodate has the disadvantage in the traditional breadmaking processes that a slight excess readily produces overmaturity. During a current investigation of some aspects of these new processes using potassium iodate as the maturing agent the authors have found that:

1. The extent of mixing of the dough determines the state of maturity once the minimal level of potassium iodate has been added, and the mixing time for optimum maturity is sharply defined (table 1).
2. As the mixing process has a greening effect on the dough (table 1), iodate sufficient to produce an initially overmature dough is required to achieve optimum maturity at the end of the process.
3. Quantities of iodate many times in excess of this requirement have little effect on the state of maturity (table 2).

4. A broad tolerance in the mixing time for optimum maturity is obtained if the iodate solution is added towards the end, instead of at the beginning of the mixing process (table 1).
5. If a short relaxation time is allowed after mixing and before addition of the iodate the effect on the bread is similar to that of shortening the mixing time, that is, there is a trend towards overmaturity.

These conclusions were reached on a series of New Zealand commercial flours, and they are illustrated by the results tabulated below. These were obtained with a typical untreated straight-run commercial flour of nominal 78% extraction.

TABLE 1—Effect of Mixing Time and Stage of Addition of Iodate on Loaf Volume and Maturity

(Loaf volumes expressed in ml per gram of flour)

*Mixing time: minutes	2	4	6	8	10	12	14	16
50 p.p.m. KIO_3 added at start of mixing	4.2 (VOM)	4.3 (OM)	5.0 (M)	4.8 (G)	4.5 (VG)	4.2 (VG)	4.3 (VG)	4.0 (VG)
50 p.p.m. KIO_3 added at end of mixing	3.9 (VOM)	4.9 (OM)	5.0 (M)	5.3 (M)	5.3 (M)	5.3 (SG)	5.4 (SG)	4.9 (G)

*The mixing times shown do not include a final 30 seconds needed to incorporate the iodate solution or its equivalent volume of water.
VOM = Very overmature; OM = Overmature; M = Mature; SG = Slightly green; G = Green; VG = Very green.

TABLE 2—Effect of Iodate Level on Loaf Volume and Maturity, at Optimum Mixing Time.

(Loaf volumes expresed in ml per gram of flour)

KIO_3: p.p.m. of flour	Nil	25	50	100	150	200	250	300	400
KIO_3 added at start of mixing	4.4 (G)	4.8 (SG)	5.2 (M)	5.2 (M)	5.1 (M)	5.1 (M)	4.9 (M)	4.9 (M)	4.9 (M)
KIO_3 added at end of mixing	4.4 (G)	4.8 (SG)	5.4 (M)	5.2 (M)	5.3 (M)	5.1 (M)	5.1 (M)	5.2 (M)	5.2 (M)

Table 2 shows the effect of iodate level at the optimum mixing times, 6 and 8 minutes for early and late additions of iodate respectively. Shorter mixing times tend to produce overmature loaves and longer mixing times green loaves, but in both cases variations in the iodate level above 25 p.p.m. have no apparent effect on the state of maturity.

The dough formula was 300 g flour, 9 g yeast in 24 ml suspension, 6 g salt, and 6 g sugar in 120 ml solution, 0·8 g glyceryl monostearate in 4 ml emulsion and 12 ml potassium iodate solution of suitable concentration (e.g. 1·25 g KIO_3 per litre $\equiv$ 50 p.p.m.). Additional water was added as required for optimum absorption, 72 ml for the flour mentioned.

Where the potassium iodate was added at the start of mixing, 12 ml of the additional water was retained and added 30 sec. before the end of mixing. Likewise when no improver was used 12 ml of water was added 30 sec. before the end of mixing.

Doughs were mixed on a Brabender farinograph, and an amount equivalent to 125 g of flour scaled off, rounded up by hand, panned, proofed one hour at 32°C and 100% relative humidity, and baked in a rotary hearth oven 20 minutes at 250°C. Loaf volumes were measured 24 hours after baking. The loaves were also judged for maturity, crumb texture, and crumb colour, and these results paralleled the volume scores.

The findings reported here are of theoretical significance in relation to the mechanism of improver action. The effects of adding the iodate late in the mixing stage and after relaxation periods are being more fully investigated.

In some countries, including New Zealand, potassium iodate may not legally be used in commercial bread production. Where it is permitted, however, the late addition of iodate may be of practical use in bakeries employing continuous dough processes, particularly where variations in flour quality and mixing tolerance occur.

A LEAF SQUASH TECHNIQUE FOR CHROMOSOME STUDIES IN GRASSES

By G. DE LAUTOUR, Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(Received for publication, 23 November 1959)

Summary

A satisfactory leaf squash technique has been developed for chromosome studies in grasses. Leaf buds, dissected from actively growing young tillers, were pre-treated in solutions of colchicine or other spindle inhibitors. After fixation, portions of leaf tissue were stained by the Feulgen method and maceration was completed by pectinase treatment. Good cell separation and staining were achieved by this method. Young leaves provided more dividing cells than did root tips.

INTRODUCTION

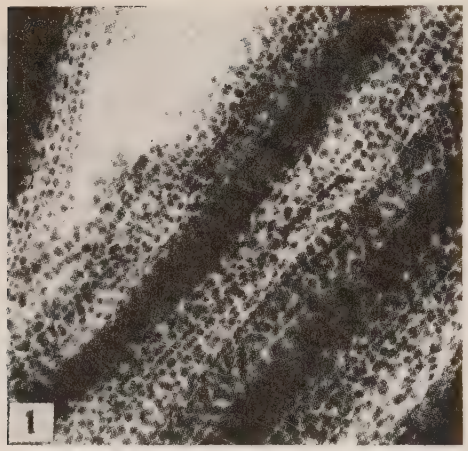
In a breeding programme at Grasslands Division involving the induction of polyploidy in sterile grass hybrids, there was a need for a simple cytological method for early assessment of the effectiveness of various colchicine treatments. Leaf buds provide the most appropriate source in which to observe colchicine effects.

Young grass leaves proved difficult to macerate (Fig. 1) and it was thought that pectinase, which had previously been found satisfactory with barley root tips by McKay and Clarke (1946), might be equally effective with young grass leaves. The method described here is based on that of Wolff and Luippold (1956) for barley root tips.

METHOD

Numerous grass species have been treated and the following procedure has been found to be satisfactory:

1. Remove the outer leaves from actively growing young tillers, using a dissecting microscope, leaving only the youngest elongating leaves and small terminal buds attached to the stems. Discard the upper portions of these leaves (which may have attained a length of 10 mm or more) leaving approximately 3 mm attached to the stems. A short portion of stem (1–2 mm) is left attached to the leaves and buds to provide a convenient "handle", for the tissues are very fragile after pectinase treatment.
2. Pre-treat for 3 hr in 0·2% colchicine solution or in saturated solutions of para-dichlorobenzene or monobromonaphthalene in water.
3. Fix overnight in 1 : 3 acetic-alcohol or 1 : 3 propionic-alcohol. This time may be shortened to a few hours, if necessary.
4. Rinse successively in 70% alcohol and 40% alcohol, and then transfer to distilled water. The buds should be allowed to sink in each solution.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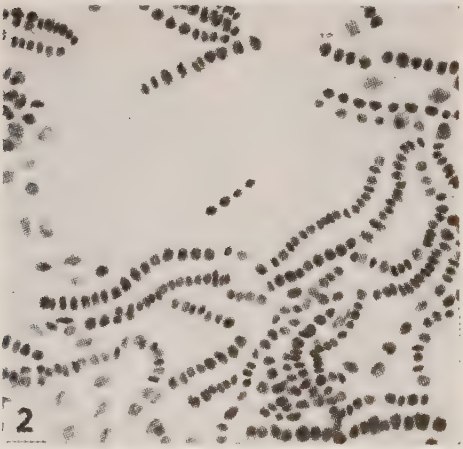
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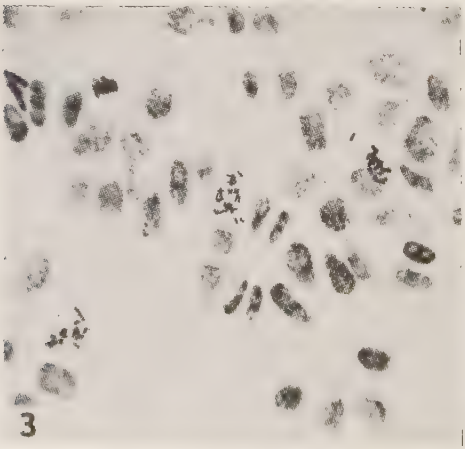
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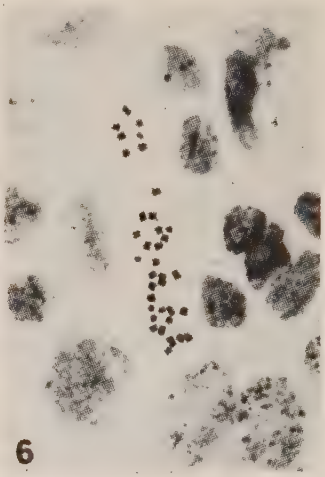
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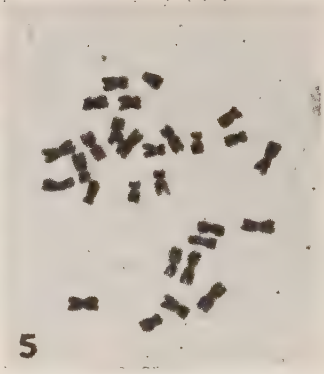
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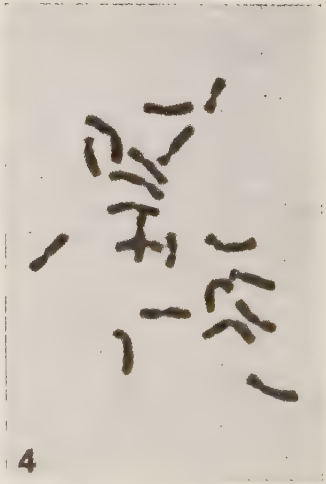
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FIG. 1—Part of young leaf of a *Lolium* hybrid, after heavy tapping. No pectinase treatment. × 140.

FIG. 2—Tissues from young leaf of a *Lolium* hybrid, after gentle tapping following pectinase treatment. × 180.

FIG. 3—Similar to Fig. 2, after further tapping. × 360.

FIG. 4—*Anthoxanthum odoratum* 2 BD fixation. 2n = 20. × 1350.

FIG. 5—*Lolium perenne* tetraploid. 2n = 28. × 1350.

FIG. 6—*Pennisetum clandestinum*. 2n = 36. × 1350.

5. Hydrolyse for 8–10 min. in normal HCl at 60°C.
6. Stain in leucobasic fuchsin (Darlington and La Cour, 1947) for a minimum of 1 hr.
7. Wash three times in distilled water, allowing 2–3 min. in each change.
8. Treat for 30 min in a 3% solution of pectinase* in a 0·1M acetate buffer at pH 3·5–4. This treatment may conveniently be carried out in the recesses of a porcelain spotting tile.
9. Transfer to distilled water.
10. Carefully squash a small piece of the leaf tissue in 45% acetic acid. Smeared coverslips may be used and the preparations made permanent by the method described by Darlington and La Cour (1947).

Method of Squashing

The small piece of leaf tissue is flooded, on a slide, with several drops of 45% acetic acid. On placing a coverslip in position a slight flattening of the tissue should be seen if maceration is adequate. While the coverslip is held at one corner the tissue is spread by gentle tapping immediately above the tissue. This forces liquid outward, carrying cells and small blocks of tissue with it (Fig. 2). Immediately no further spreading is seen, tapping should be discontinued until liquid has again flowed under the coverslip. This tapping procedure may be carried out several times until the desired degree of cell separation, as seen by microscopic examination at low magnification, is achieved (Fig. 3). As soon as this stage is reached, and before much liquid has flowed back under the coverslip, surplus liquid should be removed and the preparation gently pressed between several layers of absorbent paper. Groups of chromosomes may be further spread by local tapping with the side of a dissecting needle, gentle heating and further application of pressure. The coverslip may then be temporarily sealed with petroleum jelly or the preparation may immediately be made permanent.

DISCUSSION

Some buds showed a marked tendency to float on the surface of colchicine solution and, although penetration of the solution appeared, from the results, to be satisfactory, it was decided to commence pre-treatment under reduced pressure and observe whether or not the colchicine effect was improved. This was carried out at the beginning of stage 2. Loosely corked vials containing the buds in colchicine solution were placed in a suitable container and subjected to a vacuum of 20 mm. of mercury for 30 min., after which the vials were restored to atmospheric pressure. Gentle tapping of the vials usually resulted in the sinking of the buds. Contraction of the chromosomes brought about by colchicine pre-treatment did not appear to be improved by the vacuum treatment.

*Pectinase from L. Light and Co. Ltd., England

Where aqueous fixatives are used, such as 2BD (La Cour, 1931), the vacuum treatment may be an advantage, for floating of the buds might prevent rapid penetration of the fixative. In the procedure outlined above, this fixative, followed by hydrolysis for 20 minutes, has proved satisfactory (Fig. 4). Bleaching, where required, may be successfully carried out with alcoholic peroxide (Darlington and La Cour, 1947).

Wolff and Luippold (1956) used 4%–5% solutions of pectinase dissolved in water and adjusted to pH 4. With the sample of pectinase used a 3% solution was adequate, and it proved more convenient to dissolve the solid in an acetate buffer. By this means small quantities of solution can be prepared (e.g. 30 mg dissolved in 1 ml of buffer). Unused solutions have retained their activity after storage at 8°C for 6 days. After pectinase treatment buds may be stored for a few days in distilled water at 4°C. Prolonged storage should be avoided, however, as there is a gradual deterioration in the staining and clarity of the chromosomes. Freezing should be avoided as it causes collapse of the cells.

Prophase and early metaphase stages are sometimes rather susceptible to damage during squashing after pectinase treatment. Tandler (1959) has stated that some commercial samples of pectinase "contain proteolytic impurities which may affect the structural integrity of the chromosomes". Little trouble has been encountered, in the method described here, with metaphases which have been well contracted by pre-treatment, provided that enzyme treatment has not been excessive. The alkali-formaldehyde method of Tandler (1959) has not so far been found satisfactory with grass-leaf squashes.

Whereas it is often difficult to find dividing cells in root tips of grasses, young leaves of actively growing young tillers have been found to contain many cells in division. In a *Lolium* hybrid many dividing cells were present, both in the procambial strands and in the mesophyll of the youngest leaves enshrouding the shoot apices. Numerous cell divisions have also been found near the bases of the next longest leaves. Fewer divisions were found in the shoot apices.

The method described above has given good results with the following grasses: *Agropyron repens, Anthoxanthum odoratum* (Fig. 4), *Lolium* spp. (Fig. 5), *Lolium* spp. *X Festuca arundinacea, Pennisetum clandestinum* (Fig. 6), *Phleum* sp. and *Poa annua.*

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LIMITATIONS IMPOSED BY SOME WATERBORNE PRESERVATIVES ON THE SOIL BLOCK TEST METHOD

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(Received for publication, 12 February 1960)

Summary

With some water-borne preservatives the soil block method has been found to produce effects which limit its usefulness as a precise indicator of toxic effectiveness.

Tests have shown that the presence of soluble salts in the treated blocks, resulting either from incomplete fixation of the preservative ingredients or as by-products of the fixation reactions, affects the wood-moisture relations of the blocks.

The consequences of this are:

1. Each block creates its own micro-environment with relation to moisture, and hence blocks treated with different preservatives or even different retentions of the same preservative are not strictly comparable.
2. Weight losses from blocks showing no sign of attack can take place due to leaching during the decay test and these may obscure the threshold value.

INTRODUCTION

The tests described in this paper have been carried out to discover the reasons for certain anomalous results obtained during a recent series of soil block tests aimed at determining the threshhold values for a number of water-borne preservatives which are, or have recently been, in commercial use in New Zealand.

These preservatives were:

	Reference Abbreviation	Preservative	Formulation Components
1	CCA(1)	Copper-chrome-arsenate	CuO CrO_3 As_2O_5
2	CCA(2)	Copper-chrome-arsenate	$CuSO_4 5H_2O$ $K_2Cr_2O_7$ As_2O_5
3	CZCA	Copper-zinc-chrome-arsenate	CuO CrO_3 ZnO As_2O_5
4	FCAP	Fluor-chrome-arsenate-phenol	NaF Na_2CrO_4 $Na_2H\,AsO_4$ $C_4H_3(OH)\,(NO_2)_2$ — (DNP)
5	B.A.	Boric acid	H_3BO_3

The soil block test for evaluating wood preservatives and determining threshold values was developed by J. Leutritz Jr. of the Bell Telephone System of America and fully described by him (Leutritz, 1946) and many subsequent workers in the field of wood preservation research.

The main object of laboratory decay tests has been to provide a rapid and reliable method for studying different preservatives in relation to the following aims:

1. To compare the relative toxicity of different preservatives.
2. To obtain threshold values for such preservatives against standard test fungi.
3. To obtain a means of gauging the probable service life of such preservatives.

To accomplish these aims the method had to fulfil certain strictly defined requirements.

To compare relative toxicities it is necessary that the test method should provide reproducible environments which are such that decay will proceed rapidly and uniformly. In this respect variables such as soil type and moisture content, aeration, wood species, preservative carrier, etc., have been extensively studied and a summary of these findings has been produced by Catherine G. Duncan (1958).

In comparing the toxicity of different preservatives a visual examination of the blocks after exposure gives a good picture of relative preservative effectiveness but, for the determination of threshold values, this method is considered too variable in interpretation, and other less subjective methods must be used. At present the standard method of assessing decay is the loss of weight of blocks after exposure and this is stated either as straight weight loss, or as percentage weight loss.

Because weight losses vary from one block to another of the same retention it is necessary to have a number of replicates to obtain reliable averages which can be depicted graphically. When these weight losses are plotted against concentration a curve is obtained which should provide the precise value of the threshold, for that fungus-preservative combination, at the point where it cuts the abscissa (Fig. 1).

In practice weight losses were often found even in the highest retention group where visually there was no evidence of attack. Such losses are customarily disregarded, or, if they can be plotted on a line which intersects the decay loss line at a point on or near the zero loss ordinate, this point is taken as the threshold value (Fig. 2).

In other cases, however, these lines do not intersect at a definite point unless they are extrapolated from the more linear parts of each curve (Fig. 3) and in these cases it is difficult to assign the threshold value or to define its limits with any confidence.

To overcome this difficulty and achieve results giving a clearly defined threshold value it was considered necessary to find the causes of loss of weight in apparently sound blocks.

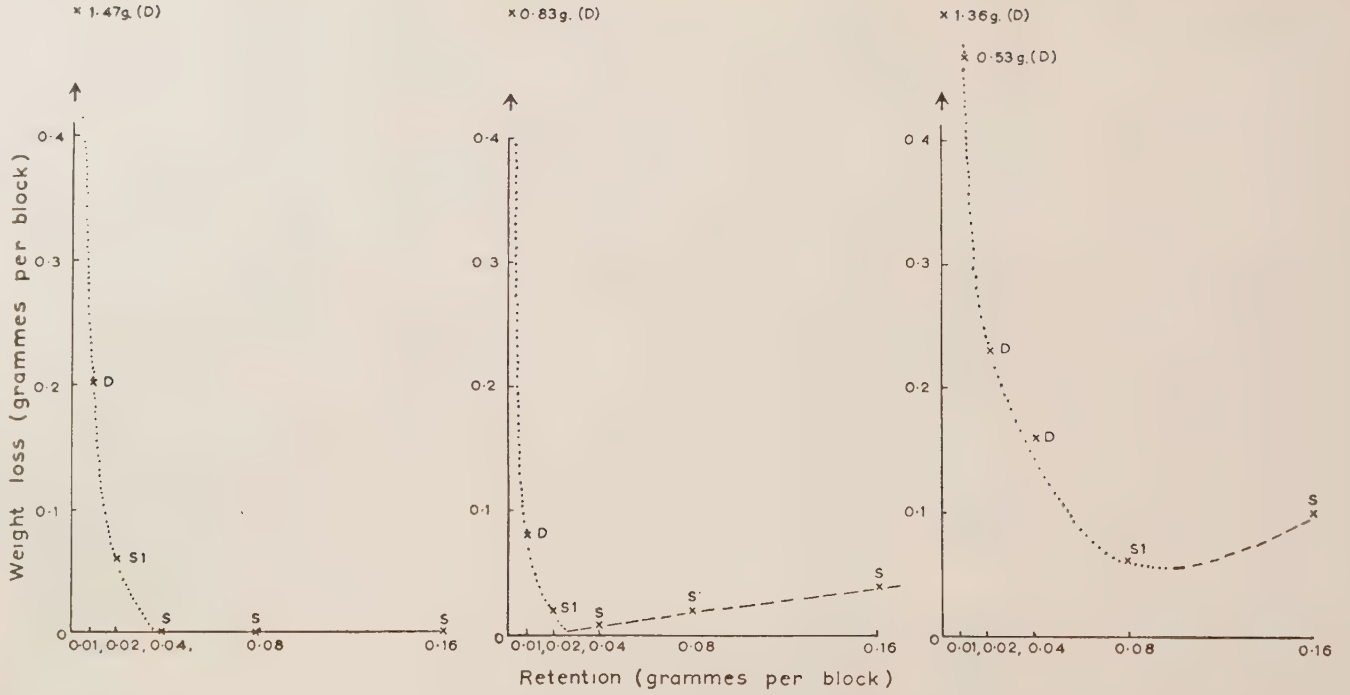

FIG. 1—Weight losses recorded for blocks treated with CCA (1) and attacked by *Poria vaporaria.*

FIG. 2—Weight losses recorded for blocks treated with CCA (2) and attacked by *Lenzites trabea.*

FIG. 3—Weight losses recorded for blocks treated with FCAP and attacked by *Poria vaporaria.*

D = decayed; Sl = slightly decayed; S = visually sound.

Various factors such as the steam sterilisation of treated blocks before exposure, and too rapid conditioning to too low equilibrium moisture content after exposure were investigated and shown to be possible causes of some weight loss in some cases (particularly with boric acid), but these did not cover all cases and losses still occurred with the most careful handling of the material.

This weight loss phenomenon has been noted by several workers including Baechler and Roth (1956) who stated: "In many cases sound blocks showed weight losses of several per cent, probably due to some leaching of preservative during the decay test".

For such leaching to take place during the decay phase it would be necessary that the moisture content of the treated blocks should rise well above fibre saturation and this has been stated by Leutritz (1946) and others not to take place normally with the soil-block technique.

Where moisture contents have been noted above this point it has generally been ascribed either to the hygroscopicity of the salt or to the metabolic processes of the fungi (Richards, 1947).

Both these factors undoubtedly have some influence but do not explain the recorded weight losses in blocks not attacked by decay and those treated with a non-hygroscopic salt.

To determine the actual moisture contents reached in treated blocks a series of tests was carried out in which all the conditions of the normal soil block test were carefully reproduced with the exception of fungal inoculation.

Test Procedures and Results

A series of standard $\frac{3}{4}$ in. sap *Pinus radiata* cubes which had been selected for uniform density were treated to retentions of 0·16, 0·08, 0·04, 0·02, 00·1 grams dry salt per block (approx. 1·4, 0·7, 0·35, 0·18, 0·09 lb/cu. ft.) with the five preservative solutions. The blocks were held under conditions of restricted drying for 3 weeks and then air dried and finally conditioned to constant weight in a humidity cabinet at 34°C and 62% RH.

Sets of 6 blocks (one of each retention plus one untreated control) were steam sterilised and placed on $\frac{1}{8}$ in. radiata pine feeder strips in standard soil block test jars.

The soil used was a fine nursery loam with a moisture content adjusted to 43% of the oven dry weight. This represented 130% of the water holding capacity of the soil as suggested most suitable in the A.S.T.M. provisional standard for the method.

The jars were held at 28°C for 28 days and then all blocks were weighed, oven dried, re-weighed and moisture contents calculated. These results are given in table 1.

To test the effect of varied soil moistures on block moisture content additional tests were carried out with the two treatments showing the greatest increase (FCAP and BA) using soil moistures of 33% and 25%. These results are shown in table 2.

TABLE 1—Moisture Contents of Treated and Control Blocks

Preservative	Retentions (grams per block)					
	0	.01	.02	.04	.08	.16
	Moisture Contents					
CCA (1)	28	30	29	29	29	29
CCA (2)	29	32	37	41	43	62
CZCA	30	30	29	30	29	30
FCAP	29	–	34	43	62	92
BA	31	30	32	34	38	79

– No block of this retention available.

TABLE 2—Block Moisture Contents at Soil M.C.% of 25% and 33%

Retention (g/block)		0	.01	.02	.04	.08	.16
Preservative	Soil M.C.	Moisture Contents					
BA	33%	29	30	31	31	39	72
BA	25%	29	30	32	36	42	82
FCAP	33%	29	–	31	35	49	87
FCAP	25%	29	–	31	41	–	83

– No blocks of these retentions available.

This series showed that even using soil moistures well below those generally advocated both preservatives caused a considerable moisture content build up in the treated blocks and that this increase was proportional to the concentration of preservative present. The results were not affected significantly by the different soil moistures within this range.

Of the preservatives used one (BA) is known to be non-fixed and one (FCAP) is only partly fixed in treated wood. The other three are all classed as highly fixed preservatives. Of these, two are formulations containing only oxides whereas the third (CCA (2)) contains K and SO_4 which do not take part in the fixation reactions and presumably remain in the wood as the soluble salt K_2SO_4.

Thus it would appear that the moisture content increase is due to the presence in the blocks of non-fixed water-soluble salts.

If this is so, removal of such salts by leaching should remove the effect and to test this an additional series was tried using blocks which had been leached for 1,000 hours in running water.

This series showed that with both preservatives tested the tendency for moisture to build up in the blocks had been removed by the leaching procedure.

Table 3—Moisture Contents in Leached Blocks: Soil M.C. 43%

Preservative	0	.01	.02	.04	.08	.16
CCA (2)	31	30	30	29	30	30
FCAP	29	30	29	29	30	30

Boric acid was not tested in this series as the leaching procedure had been shown chemically to have removed all the salt present.

In considering the reason for the moisture increase it is obvious that the moisture must come either from the atmosphere in the jar or by liquid movement from the soil itself via the feeder strip. It has been pointed out previously by Harrow (1951) that capillarity must be ruled out as the untreated controls are not affected. This however does not rule out the possibility of moisture diffusion proceeding to a point where osmotic pressures could be set up by the soluble salts, with the wood itself acting as a semi-permeable membrane.

To test the method of uptake unleached blocks treated with FCAP and BA were supported on glass rods well clear of the soil surface. (This was done by placing a watch glass on the feeder strip and laying glass rods across the rim of the watch glass.)

The moisture contents of these blocks after 28 days are given in table 4.

Table 4—Moisture Contents of Blocks Supported Clear of the Soil

Preservative	0	.04	.08	.16
FCAP	28	34	47	67
BA	29	31	35	62

These results show slightly lower values than when the blocks were placed directly on the feeder strips but prove that the majority of the moisture taken up undoubtedly comes from the atmosphere.

Although some of the preservatives and component salts used in these tests are not considered hygroscopic at normal humidities, it has been shown (G.B.D.S.I.R., 1958) that some salts (e.g. sodium chloride) have a critical relative humidity only above which do they pick up atmospheric water. For NaCl this RH is 70%. As neither potassium sulphate nor boric acid would normally be considered as hgyroscopic as sodium chloride it was decided to test the RH% actually found in soil jars. This was done by measuring the RH% of the atmosphere over soils of varied moisture contents using a Lambrecht stem hygrometer. The results of this test are shown in table 5.

Table 5—The Relative Humidity of Atmospheres Over Soils of Varied M.C.%

Soil M.C.%	0	5	15	25	33	43	50
R.H.%	13	91	93	92	93	93	93

Within the limits of the instrument used, this test showed that the atmosphere within the jars closely approached saturation and was little

affected by changes in soil moisture contents above 5% of the oven dry weight. Under normal laboratory conditions the equilibrium moisture content of the soil used is 2–3% and it would appear that so long as soil moisture is above the equilibrium value for the incubator temperature enough moisture will be provided to maintain the atmosphere at near saturation level.

The exact mechanism of the uptake is not known but presumably it is either that the very high humidities induce hygroscopicity in all the salts under consideration or that some moisture condenses on the surfaces of the blocks setting up osmotic pressures which cause the moisture to move progressively inwards.

Although this moisture build up was noted by Harrow (1951) with zinc chloride, he considered that diffusion of the salts from the blocks would not take place due to the absence of capillary movement.

Examination of feeder strips after the test period however show that this does take place. Feeder strips from jars containing 6 boric acid treated blocks (one of each retention plus control) showed overall moisture contents of 70% and boric acid loadings of over 0·3%.

The diffusion of salts can also be readily seen with feeder strips under high retentions of fluor-chrome-arsenate-phenol. The bright yellow of the dinitrophenol is clearly visible under such blocks, and unless the fungal growth has become firmly established before the blocks are introduced decay, of these portions of the feeder strips is halted.

Relating the weight losses to the original salt concentration shows that with FCAP up to 56% of the original preservative present can be lost due to diffusion removal during the 12 weeks the blocks are normally in the decay jars. This denotes a fairly low degree of salt fixation. With CCA (2) weight losses of sound blocks of up to 24% of the original salt were found. This is within the theoretical amount of K_2SO_4 produced by the fixation reactions (i.e. 28%).

CONCLUSIONS

The presence of soluble salt in the treated blocks causes a significant rise in the moisture content of these blocks during the period of exposure to the test fungi. In some cases the moisture content can increase to such a point (110% in one block examined) that fungal attack could be inhibited solely by the decrease in aeration brought about by high moisture contents. (Leutritz (1946) found the following limiting moisture contents at which no decay took place – "78% for *Poria incrassata;* 84% for *Coniophora cerebella;* 66% for *Polyporus vaporarius*").

The increase apparently takes place over a wide range of soil moisture contents, in fact, any moisture content which is high enough to remain above the soil, e.m.c. for the duration of the test.

With this rise in moisture content diffusion of the soluble salt will proceed from the treated block to the untreated feeder strip resulting in a significant weight loss in that block irrespective of fungal attack. If the fungus has not become sufficiently well established before the treated blocks are

introduced the rapid migration of some preservatives may halt its development and thus protect otherwise vulnerable blocks in the same jar. (This is the case where several retentions are placed together in the one jar – not standard practice but a good method of reducing between jar variability for various retentions of well fixed, non volatile preservatives.)

Where the weight loss due to decay grades smoothly into that due to preservative leaching it is impossible to determine the threshold value accurately.

With leached blocks it is possible to define the threshold precisely but this procedure is not satisfactory for any preservative which does not achieve a relatively high degree of fixation.

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THE ALPINE GRASSLANDS OF THE HOKITIKA RIVER CATCHMENT, WESTLAND

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(Received for publication, 28 January 1960)

Summary

In the alpine grasslands of the Hokitika river catchment there are ten distinct associations. Three of these, characterised by dominance of *Poa colensoi, Rostkovia gracilis,* and *Danthonia setifolia,* appear to have arisen as a result of heavy grazing by deer and/or chamois and/or hares, while a further association, *Carpha alpina - Oreobolus pectinatus* is a peat-bog association of restricted extent. The remaining six associations, characterised by dominance of *Danthonia rigida, D. flavescens* (broad-leaved form), *D. flavescens* (narrow-leaved form), *D. crassiuscula, D. oreophila,* and *Poa cockayniana* are of wide distribution.

A table summarises the habitat factors underlying this distribution of associations.

All associations are modified by grazing, but to very varying extents. A table gives examples of specific frequencies of each association for differing amounts of grazing modification.

The present condition of the associations as revealed by study of the data collected at 43 permanent sampling points varies from poor to excellent and there is no uniformity in direction of trend in condition of the associations.

The incidence of accelerated erosion is generally low wherever soils are derived from schists (though with a few "sore-spots") but is substantially higher on greywacke terrain. Control of animals is necessary for relief of pressure on these schist area "sore-spots" and the greywacke lands.

INTRODUCTION

The Hokitika River and its major tributaries, the Styx, Toaroha, Kokatahi, and Whitcombe rivers, drain an area of mountain land of high relief, approximately 154,000 acres in extent (Fig. 1). The watersheds rise to a height generally exceeding 5,000 ft, reaching 7,000 ft on the main divide and 8,500 ft in the extreme south. The rivers are true alpine torrents, descending steeply through deep valleys and narrow gorges to the coastal plain. These valleys and gorges are filled with heavy forest, above which subalpine scrublands extend upward to a height of 3,500–4,500 ft. Above the scrublands, which attain their maximum altitude on the steepest slopes (Fig. 2), there are extensive alpine grasslands, some grassland persisting upslope to an altitude of 6,200 ft though, above 5,000 ft, the grassland is much broken up by barren rock outcrops, stone-fields and active screes

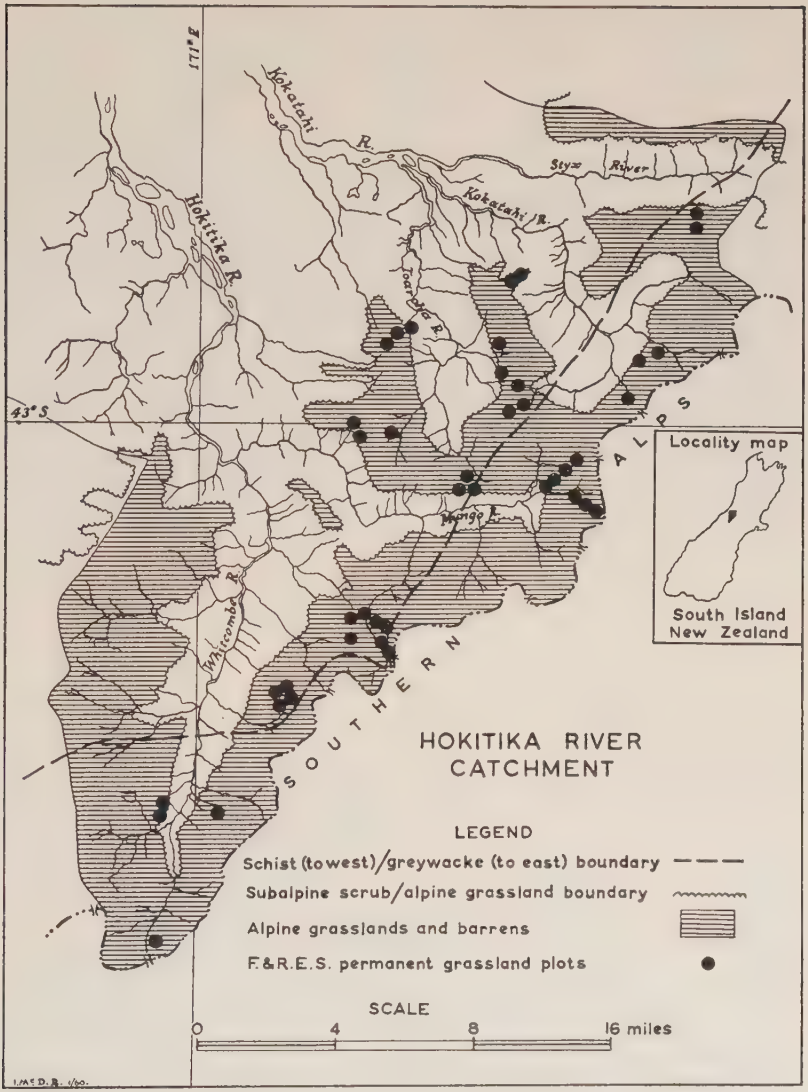

FIG. 1—Locality map and detail of Hokitika river catchment.

(Fig. 3). Permanent snow-beds, snow-fields, and small glaciers lie on the highest peaks and, in heavy snow years, snowbeds may last throughout the year within the grassland zone. In all, the alpine grasslands and alpine barrens comprise approximately one half the total area of the river catchments, the grasslands exceeding the barrens several times in extent.

Photo—P. Wardle.

FIG. 2—Showing the boundary between subalpine scrub and alpine grassland; subalpine scrub going higher on the steeper slopes.

FIG. 3—The upper limit of alpine grassland abutting rock and permanent snow.

No satisfactory botanical or ecological account of these grasslands has yet been given, despite their obvious importance in the field of watershed management. The slopes they occupy are steep to very steep, averaging 25°–40°, increasing to more than 50°. Soils are shallow, weak-structured, weakly-weathered soils derived from schist and greywacke rocks of low erosion resistance. The climate is severe. No accurate data are available but annual rainfall probably exceeds 300 in. with periodic, prolonged, high-intensity falls. Frost action is pronounced with daily freeze and thaw in clear weather, even in mid-summer. Gale force winds are frequently experienced. Loss or weakening of the ground cover leads rapidly to complete loss of the soil and to exposure of the weak bed-rock to frost shatter and subsequent rapid transport into stream channels by gravity and water.

Red deer have been present in the area for 50 years (Thomson 1922), and chamois for approximately 25 years (Wodzicki 1950), populations of these animals reaching a peak around 1945–50. Hares are present throughout, particularly on greywacke terrain near to the main divide. Opossums range up into the grasslands from the scrublands and forests though populations are low except in mosaic areas of scrub and grass. Therefore, though these grasslands have never been extensively used by domestic animals (brief attempts to use them for the grazing of sheep were made between 1890–1900), and though there is little evidence of fire (some fires may have been set by the sheep-men or by gold-prospectors and there have been minor accidental fires), they are by no means virgin grasslands.

This paper, based on studies carried out as part of an examination of the condition of the Hokitika river watersheds by staff of the Forest and Range Experiment Station, New Zealand Forest Service, during the summer 1957–58, is an account of the grasslands as they are, degrees of modification and current trends being briefly indicated.

METHODS

Permanent sample plots were established at 43 sites, selected, after extensive reconnaissance, as typical of the most important grassland associations present. Each plot consisted of a line transect, the length of line being normally 200 links, reduced in a few instances to 100 links in precipitous country. Along each transect, specific frequency (Stapleton 1913) of all species down to mosses was recorded using a six-inch-diameter ring at stations 2 links apart. Point cover (vegetation, litter, bared soil, bare rock, erosion pavement, etc.) was recorded at the mid point of each station. Supplementary notes for each transect provide detail concerning aspect, altitude, soil profile, intensity of animal use, species grazed, etc. Photographs of each plot and its surrounds, mainly in colour, were taken whenever weather conditions permitted. The position of each plot is pin-pointed photographically and marked on the ground by steel rods. Analysis of plot data has been in the light of records made on more general reconnaissance work.

THE PLANT ASSOCIATIONS AND THEIR HABITATS

Six major and four minor associations ([1]) were recognised, each characterised by the occurrence of one or more particular physiognomic dominants ([2]).

1. *Danthonia rigida* (red tussock) Association

This association is normally restricted to gentle slopes below 4,000 ft altitude on schist terrain. Soils are deep and poorly drained. The association is invariably contiguous with subalpine scrubland and contains many shrub species. *D. rigida* is also the normal dominant on the small frost-flat grassland pockets in the river valleys (altitude 1,000–2,000 ft) but otherwise the two associations are distinct. The valley grassland areas will not be further considered. They are of insignificant total extent.

2. *Danthonia flavescens* (t.b.l.) ([3]) Association

The association occurs on slopes of any aspects, principally at about 4,000 ft altitude and on slopes approximating 30°. Soils are typically shallow, skeletal and well drained. The association is more widely developed on greywacke than on schist terrain.

3. *Danthonia flavescens* (s.n.l.) Association

This association also occurs on slopes of any aspect but is most widely developed at about 4,500 ft altitude. The angle of slope is more varied, from less than 20° to more than 40°, but this is probably only a reflection of the overall distribution of topography classes at this altitude. The association occurs more widely on schist than on greywacke country. Soils are deeper and appear relatively older than the soils under *D. flavescens* (t.b.l.), drainage again being very good.

4. *Danthonia crassiuscula* Association

Grassland with *D. crassiuscula* dominant is most widely developed at altitudes greater than 4,500 ft and extends to the limits of vegetation. Where it occurs at lower altitudes, the sites occupied are those where snow lies late in the season. Above 4,500 ft, sunny aspect slopes are favoured, the angle of slope rarely exceeding 35°. Soils are generally strongly skeletal and stony. Greywacke or schist.

5. *Danthonia oreophila* Association

This association occupies a very clearly defined habitat, namely sites where the soil is very shallow and well drained, particularly ridge crest sites where bedrock is near to the surface. Altitude range from 4,500 ft to vegetation limits, slopes generally below 30°, schist or greywacke terrain.

([1]) The term "association" is here used in a general sense, implying no more than that the plant grouping concerned is distinct and clearly recognisable on the ground.

([2]) Species names are those given in Cheeseman (1925) with the exception noted in the appendix to this paper (q.v.).

([3]) *Danthonia flavescens* (t.b.l.) and *D. flavescens* (s.n.l.) signify respectively, the tall broad-leaved and the short narrow-leaved forms of this species.

6. *Poa cockayniana* ([4]) Association

P. cockayniana achieves dominance on the rocky mixed soils of avalanche pathways at altitudes below 4,300 ft. Some shrub species are normally present.

7. Minor Associations

Danthonia setifolia, Poa colensoi, and *Rostkovia gracilis* are found as dominants on a variety of sites at or about 5,000 ft altitude, site factors being similar to those of the *D. crassiuscula* association. These three minor associations are considered to be induced by grazing, mainly by modification of *D. crassiuscula* swards. The fourth minor association is characterised by dominance of *Carpha alpina* and *Oreobolus pectinatus.* The association is restricted to areas of deep, peaty, and poorly drained soil on level to near-level ground at altitudes within the subalpine scrubland zone. It frequently merges into *Danthonia rigida* grasslands and contains tussocks of this species. There is a possibility that a fifth minor association was once represented, *Danthonia cunninghamii* dominant, on damp shady sites below 4,500 ft but this appears to have been replaced, under grazing pressure, by one or other of the *D. flavescens* associations.

Habitat factors for plots established in these various associations are summarised in table 1.

THE COMPOSITION OF THE ASSOCIATIONS

As already noted, the grasslands of the Hokitika river catchment have already undergone extensive modification through the grazing of deer, chamois, and hares. This modification has been in the same direction as that known for all other natural grasslands when subjected to animal pressure . . . high-producing English grassland (Jones 1933), low-producing dense annual grassland in California (Talbot *et al.* 1939), mid-west U.S. rangelands (Dyksterhuis 1952), or sand-dunes, salt-meadow, sub-maritime and various dense annual grasslands in New Zealand (Wraight 1957). Patterns of modification with grazing to different intensities, for the subalpine grasslands of the Hokitika, must be assumed to be broadly comparable. In accordance with these known patterns, therefore, the Hokitika plots have been grouped into three classes – slightly modified, strongly modified, and very strongly modified, according to the "degree of departure from climax" (Dysterhuis *loc. cit.*). An example of each association, in its least and in its most modified state is given (see table 2).

1. *Danthonia rigida* Association

Both plots established in this association are classed as strongly modified. *Danthonia rigida* forms a part open canopy. Characteristic associate species are the shrubs *Dracophyllum uniflorum,* various small-leaved *Coprosma* species, *Suttonia nummularia,* and the fern *Meringium multifidum.* These are all species of the adjacent subalpine scrubland. Other species present are those of the *Danthonia flavescens* (t.b.l.) association. It is reported (Mr N. L. Elder, *pers. comm.*) that in the Southern Ruahine Range, *D. flavescens*

([4]) *Poa cockayniana* and *Danthonia setifolia* were misnamed *P. anceps* and *D. buchanani,* respectively, in the (unpubl.) interim report on the Hokitika river catchment by the Forest and Range Expt. Station, New Zealand Forest Service.

TABLE 1—Showing Habitat Factors of the Different Associations of the Hokitika Alpine Grasslands. Figures are Numbers of Plots in Each Habitat Factor Class

		D. rigida	*D. flavescens* Tall and Broad Leaved	*D. flavescens* Short and Narrow Leaved	*D. crassiuscula*	*D. oreophila*	*P. cockayniana*	*D. setifolia*	*Poa colensoi*	*Rostkovia gracilis*
ALTITUDE	3,000–3,500 ft	1	–	–	–	–	–	1	–	–
	3,500–4,000 ft	1	6	3	–	–	2	–	–	–
	4,000–4,500 ft	–	3	10	–	–	1	–	–	–
	4,500–5,000 ft	–	2	5	2	3	–	–	–	–
	> 5,000 ft	–	–	1	1	3	–	–	1	1
SLOPE	< 20°	2	–	2	–	2	–	–	–	–
	20–25°	–	2	1	1	1	1	1	–	1
	25–30°	–	4	6	1	3	–	–	1	–
	30–35°	–	3	4	–	–	–	–	–	–
	35–40°	–	–	3	1	–	1	–	–	–
	> 40°	–	2	2	–	–	1	–	–	–
ASPECT	N	1	3	7	1	1	–	–	–	–
	E	–	3	2	1	1	–	1	–	–
	S	1	2	6	–	2	–	–	–	1
	W	–	3	4	1	2	3	–	1	–
SOIL—PARENT MATERIAL	Schist	2	4	13	2	3	1	–	1	1
	Greywacke	–	5	5	1	3	2	1	–	–
	Schistose/greywacke	–	2	1	–	–	–	–	–	–

WEATHERING	..	Strong	..	..	–	2	6	1	1	–	–	–	–
		Weak	..	..	–	7	6	1	5	3	1	1	1
LEACHING	..	Strong	..	..	–	2	1	–	1	–	–	–	–
		Weak	..	..	–	6	5	–	2	2	–	1	–
A AND B HORIZONS	..	Deep	..	..	2	5	10	1	1	–	–	–	1
		Shallow	..	..	–	6	4	–	4	3	1	1	–
LITTER DEPTH	..	Deep	..	..	1	5	2	–	–	1	–	–	–
		Shallow	..	..	1	6	11	1	2	2	1	–	1
		None	..	..	–	–	4	1	3	–	–	1	–
DRAINAGE	..	Poor	..	..	2	–	1	–	1	–	–	–	–
		Good	..	..	–	11	18	3	5	3	1	1	1
					2	11	19	3	6	3	1	1	1

TABLE 2—Showing Specific Frequency (6-in.-diameter ring) of Various Alpine Grassland Plots in the Hokitika Catchment

	(a) *D. rigida* Alpine Grassland Strongly Modified	(b) *D. rigida* Alpine Grassland Strongly Modified	(c) *D. flavescens* (t.b.l.) Grassland Slightly Modified	(d) *D. flavescens* (t.b.l.) Grassland Strongly Modified	(e) *D. flavescens* (s.n.l.) Grassland Slightly Modified	(f) *D. flavescens* (s.n.l.) Grassland Very Strongly Modified	(g) *D. crassiuscula* Grassland Slightly Modified	(h) *D. oreophila* Grassland Strongly Modified	(i) *P. cockayniana* Alpine Grassland Slightly Modified
Danthonia cunninghamii	–	–	–	–	2	–	–	–	2 (young)
D. rigida	30	67	–	–	–	–	–	–	–
D. flavescens (t.b.l.)	39	14	100	94	–	–	–	22	10
D. flavescens (t.b.l.) young and seedling	–	–	–	–	–	–	–	–	10
D. flavescens (t.b.l.) stump	–	–	–	–	–	–	–	–	2
D. flavescens (s.n.l.)	–	–	–	–	90	58	5	–	–
D. crassiuscula	–	–	–	–	–	4	71	2	–
D. crassiuscula seedling	–	–	–	–	–	–	1	–	–
D. oreophila	–	–	–	–	–	–	–	74	–
D. pilosa	9	–	–	–	–	–	–	–	–
Poa colensoi	48	4	–	60	42	14	40	50	86
P. imbecilla	–	–	–	–	–	–	–	–	2
P. sclerophylla	–	–	–	–	4	–	–	–	–
P. cockayniana	–	–	–	–	–	–	–	–	26
P. mackayii	–	–	20	–	–	–	–	2	–
Petriella colensoi	9	–	–	10	14	2	8	18	2
Deyeuxia forsteri	–	–	–	2	–	–	–	–	4
D. avenoides	–	–	–	–	12	–	–	–	–
Hierochloe redolens	–	–	–	–	22	–	–	–	6
Unidentified small grass	–	21	–	–	–	–	–	–	–
Schoenus pauciflorus	20	42	–	30	42	–	38	–	2
Luzula campestris	8	–	–	26	6	–	13	–	2
L. colensoi	–	–	–	–	–	–	–	6	–
Rostkovia gracilis	–	–	–	4	–	2	–	10	–
Carpha alpina	–	21	–	–	–	24	–	–	–

Carex spp.	–	–	–	–	18	13	–	4	50	–	–	–
Astelia cockaynei	–	–	–	–	2	–	–	–	–	–	–	–
A. linearis	–	6	–	–	–	–	3	–	–	–	–	–
Celmisia armstrongii		33	–	–	2	16	–	–	–	–	–	–
C. gracilenta	–	20	–	–	–	–	–	–	–	–	–	–
C. petiolata	–	–	–	–	4	–	–	–	–	–	–	–
C. coriacea	–	–	–	–	12	2	–	–	–	–	–	–
C. spectabilis	–	3	–	–	–	–	–	–	–	–	–	–
C. walkeri	1	–	–	–	–	–	1	40	–	–	–	–
C. novae-zealandiae	3	–	–	–	–	–	–	–	–	–	–	–
C. incana	–	15	–	–	–	–	–	–	–	–	–	–
C. discolor	–	–	–	12	–	34	64	–	–	–	–	–
C. dubia	–	–	–	10	–	–	5	–	–	–	–	–
C. sessiliflora	–	–	–	–	–	2	27	38	–	–	–	–
Raoulia grandiflora	–	–	–	–	–	–	3	14	–	–	–	–
Senecio bellidioides	11	6	–	26	–	20	12	6	–	–	–	–
S. scorzoneroides	–	–	–	–	4	–	–	–	–	–	–	–
S. lautus	–	–	–	–	–	–	–	–	4	–	–	–
Cotula squalida	12	–	–	–	–	–	4	–	48	–	–	–
C. pyrethrifolia	–	–	–	–	–	–	–	–	12	–	–	–
Craspedia uniflora	1	–	–	–	–	2	–	–	–	–	–	–
Helichrysum bellidioides	–	–	–	78	8	–	–	–	14	–	–	–
Abrotanella linearis	–	–	–	–	–	–	2	–	–	–	–	–
Leucogenes grandiceps	–	–	–	–	–	–	–	–	2	–	–	–
Olearia ilicifolia	–	–	–	–	–	–	–	–	10	–	–	–
**Crepis capillaris*	–	–	–	–	–	–	–	–	2	–	–	–
Suttonia nummularia	–	13	–	–	–	8	–	–	–	–	–	–
Dracophyllum uniflorum	11	5	–	–	–	10	–	–	–	–	–	–
D. longifolium (seedling)		1	–	–	–	–	–	–	–	–	–	–
Pentachondra pumila	–	11	–	–	40	–	–	–	–	–	–	–
Cyathodes pumila	–	–	–	–	–	–	12	–	–	–	–	–
Gaultheria depressa	4	–	–	6	–	14	31	18	–	–	–	–
G. rupestris	–	–	–	–	–	2	2	–	–	–	–	–
Anisotome aromatica	57	29	38	76	68	50	60	82	–	–	–	–
A. pilifera	–	–	–	4	–	–	1	–	–	–	–	–
Angelica decipiens	–	–	–	–	–	–	5	–				

TABLE 2—Showing Specific Frequency (6-in.-diameter ring) of Various Alpine Grassland Plots in the Hokitika Catchment—*continued*

	(a) *D. rigida* Alpine Grassland Strongly Modified	(b) *D. rigida* Alpine Grassland Strongly Modified	(c) *D. flavescens* (t.b.l.) Grassland Slightly Modified	(d) *D. flavescens* (t.b.l.) Grassland Strongly Modified	(e) *D. flavescens* (s.n.l.) Grassland Slightly Modified	(f) *D. flavescens* (s.n.l.) Grassland Very Strongly Modified	(g) *D. crassiuscula* Grassland Slightly Modified	(h) *D. oreophila* Grassland Strongly Modified	(i) *P. cockayniana* Alpine Grassland Slightly Modified
Aciphylla trifoliata	–	–	–	2	32	–	6	8	–
A. monroi	–	–	–	2	6	–	–	–	–
Ourisia macrocarpa	1	12	4	–	4	–	–	–	6
O. caespitosa	–	–	–	28	–	–	1	–	–
Euphrasia monroi	–	–	–	2	–	–	2	–	–
Hebe sp.	–	–	–	2	–	–	–	–	–
Parahebe bidwillii	–	–	–	–	–	2	–	2	–
Coprosma pseudocuneata	3	4	–	–	–	–	–	–	–
C. repens	–	4	–	–	–	–	–	–	–
C. colensoi	–	1	–	–	–	–	–	–	–
C. petriei	–	–	–	18	–	–	2	–	–
C. propinqua	–	–	–	–	–	–	1	–	–
Nertera depressa	–	1	–	–	–	–	–	–	–
Colobanthus crassifolius	–	–	2	–	–	–	–	4	–
Cerastium sp.	–	–	6	–	–	–	–	–	–
Claytonia australasica	–	–	2	–	–	–	–	–	2
Cardamine bilobata	–	–	12	–	6	–	–	–	24
Epilobium linnaeoides	8	–	4	6	30	–	–	4	72
Pratia angulata	6	–	30	–	38	2	–	–	–
Wahlenbergia albomarginata	–	–	–	–	2	–	1	–	–
Caltha novae-zealandiae	1	–	–	40	–	14	32	30	–
Ranunculus lyallii	–	–	2 (seedlings)	–	2	–	–	–	–
R. tenuicaulis	–	–	2	–	–	–	–	–	–
Geranium microphyllum	–	–	2	–	–	–	–	–	–

Oxalis lactea	–	–	–	28	–	–	–	–	
Gentiana sp.	–	–		–	4	–	5		
Plantago brownii	–	–	–	–	–		3		–
Gunnera sp.	–				6	–			
Donatia novae-zealandiae	–	–	–	–		4	3	–	–
Phyllachne clavigera			–	–	–	–	6		
Drapetes dieffenbachii	–	–	–		–		5	–	–
Geum parviflorum	–	–	–	–			–		8
Acaena sanguisorbae	–	–	–	–	–	–	–		4
Carmichaelia grandiflora	–	–	–	–		–			2
Lycopodium fastigiatum	–	–		6			15	–	–
Chiloglottis cornuta	1	18	–	–	–	–	–	–	
Meringium multifidum	1	6	–		–	–	–		
Mecodium demissum	–	–	–	–	–	–	6	–	–
Blechnum penna-marina	1	–	–	22				–	–
Polystichum vestitum	–	–	2		2		–	–	–
Moss (various spp.)	6	50	10	2	42	10	19	36	64
Marchantia sp.	–	–	2	–	–	–	–	–	

and *Olearia* spp. "appear to coexist, young shrubs shading out the tussock, but tussock invading the older bushes as they open out". *D. rigida* grassland in the Hokitika probably plays a similar part with respect to the adjacent and contained shrubs. Figs. 4 and 5 illustrate these points.

2. *Danthonia flavescens* (t.b.l.) Association

The specific frequency data shown in column (c), table 2, are for a plot in grassland very slightly modified by grazing. *D. flavescens* (t.b.l.) forms a complete dense canopy overshading a small number of associate species which, in their etiolated state, would be particularly susceptible to grazing if they could be found by animals (see Fig. 6). The main associate grass is the upright-growing, grazing-susceptible *Poa mackayi*, not the grazing-induced *Poa colensoi* common in modified *D. flavescens* grasslands. It is also significant that there is not one composite contained in this sward.

On the other hand, in column (d), table 2, specific frequency data for a strongly modified portion of *D. flavescens* (t.b.l.) grasslands are presented. *D. flavescens* provides a sparse and open canopy (Fig. 7). All associate species are strongly resistant to grazing with the exception of *Aciphylla trifoliata* and *Anisotome pilifera* which, however, are present in very small amounts and in the protection of the taller tussocks. 'Increaser' and 'invader' species are strongly represented, notably *Poa colensoi* and the various composites.

FIG. 4—*Danthonia rigida*, alpine grassland, strongly modified, showing open canopy of *D. rigida* and shrub/tussock replacement or "co-existence".

(Plot of column (a), table 2)

Photo—P. Wardle.

FIG. 5—*Danthonia rigida* alpine grassland, strongly modified, showing open canopy and peaty soil eroded by animal tracking as well as overgrazing.
(Plot of column (b), table 2)

3. *Danthonia flavescens* (s.n.l.) Association

A slightly modified plot is represented in column (e), table 2; a very strongly modified plot in column (f). Where the sward is slightly modified, the individual tussocks are of good stature, forming a moderately well closed canopy, beneath which there is a varied assemblage of grass and herb species moderately susceptible to grazing. In the very strongly modified sward, on the other hand, the tussocks are reduced in height, sparse, and do not form a

FIG. 6—*Danthonia flavescens* (t.b.l.) very slightly modified showing complete and dense canopy afforded by dominant.

(Plot of column (c), table 2)

FIG. 7—*Danthonia flavescens* (t.b.l.) strongly modified showing scattered tillers only, of dominant.

(Plot of column (d), table 2)

closed canopy. Many species susceptible to grazing have been reduced or eliminated. Species present are mainly resistant species or invaders (e.g. *Celmisia discolor* and the low-growing woody plants *Suttonia nummularia, Dracophyllum uniflorum,* and *Pentachondra pumila*). Figs. 8 and 9 illustrate these two plots.

4. *Danthonia crassiuscula* Association

Plots established in *Danthonia crassiuscula* grassland were all in swards that are slightly to moderately modified. Specific frequency data are therefore presented for only one plot – a typical slightly modified sward (column (g), table 2). The dominant species, *D. crassiuscula,* forms a sward of variable density. It can provide a complete canopy at a height above the ground of twelve inches, but elsewhere single, widely spaced tillers occur through a turf of other species (Fig. 10). The associate grasses and herbs are in general those found in the *D. flavescens* associations except that a few species, e.g. *Celmisia sessiliflora* and *Raoulia grandiflora,* occur commonly in slightly modified *D. crassiuscula* grassland but are common in *D. flavescens* grassland only where this is strongly to very strongly modified. In virgin or slightly modified *D. flavescens* grassland, such species are shaded out.

5. *Danthonia oreophila* Association

All *D. oreophila* grassland seen was considered to be strongly to very strongly modified. An example of a strongly modified sward is given in column (h), table 2. See also Fig. 11.

D. oreophila is the most abundant species, over-topping all others with the exception of *D. flavescens, D. crassiuscula,* and the larger plants of *Celmisia walkeri.* It forms extensive mats, the leaf tips being about four inches above the ground. The *Poa colensoi* present is, at this altitude, invariably of dwarf stature, normally less than one inch high. The rush, *Rostkovia gracilis* is invariably an important associate species, as also are *Celmisia sessiliflora* and *Raoulia grandiflora.* The *D. oreophila* association is the most generally modified of all grassland associations in the Hokitika river catchment, the reason probably being that the dry ridge crests on which it occurs are strongly favoured by deer, chamois, and hare during the few months of the year these sites are snow-free.

6. *Poa cockayniana* Association

Specific frequencies for this association are given in column (i), table 2. See also Fig. 12. Associate species are mainly early seral species such as *Acaena sanguisorbae, Epilobium linnaeoides, Senecio lautus, Cotula squalida, C. pyrethrifolia,* and *Helichrysum bellidioides.* There is always much moss and, at low altitudes, much *Hypolepis millaefolium.*

FIG. 8—*Danthonia flavescens* (s.n.l.) slightly modified showing tussocks of good stature forming a moderately good canopy.
(Plot of column (e), table 2)

Photo—W. J. Wendelken.

FIG. 9—General view of *Danthonia flavescens* (s.n.l.) association on schist terrain (Plot of column (f), table 2, is in top left of picture)

FIG. 10—*Danthonia crassiuscula* association (moderately modified) showing the incomplete canopy formed by the scattered tillers of the dominant.
(Plot of column (g), table 2)

FIG 11—*Danthonia oreophila* association (strongly modified) showing the extensive mats of *D. oreophila* and occasional tillers of *D. flavescens* and *D. crassiuscula.* (Plot of column (h), table 2)

Large numbers of seedling *Danthonia flavescens* and *D. cunninghamii* are present, more so than in any other alpine grassland association in the Hokitika river catchment, and the *Poa cockayniana* association may be regarded as a seral stage leading toward ultimate development of *D. flavescens* (t.b.l.) and/or *D. cunninghamii* grassland being held in the seral stage through repeated avalanche destruction. There is also a distinct possibility that *Poa cockayniana* grassland may, at times, be derived through overgrazing of *D. flavescens* or *D. cunninghamii* swards, with return of these species after relief of grazing pressure. The present plot may be an instance of this rather than representative of a true avalanche path association.

7. Minor Associations

The dominants of these associations, *Danthonia setifolia, Poa colensoi,* and *Rostkovia gracilis,* are species of very wide distribution throughout the alpine grasslands of the Hokitika river catchment. They are thus species of wide habitat tolerance. This is a characteristic of "invader" and "increaser" species throughout the grasslands of the world (Dyksterhuis 1952). The three species are completely resistant to grazing, suggesting, therefore, that they now form pure associations where the original cover has been destroyed by overgrazing yet, because sites and soils were intrinsically stable, erosion has not been active.

The three associations are found mainly on sites within the range of *Danthonia crassiuscula* though *D. setifolia* was found to be dominant in one locality within the subalpine scrubland zone, in an area favoured by deer and chamois, and may have developed following the elimination of scrubland by overgrazing. Associate species in these three minor associations are mainly those found in strongly modified *Danthonia crassiuscula* swards. Associate species of the *Carpha-Oreobolus* association are mainly species of wet *Danthonia rigida* grassland.

GRAZING MODIFICATION IN RELATION TO WATERSHED PROTECTION

With respect to the alpine grasslands of the Hokitika river catchment, it is generally true to say that, with increasing grazing modification of the swards, the grassland becomes more open in its upper layers, there is marked reduction in the stature of the dominants, and the lower layers become increasingly composed of species growing closely appressed to the ground. In other words, the efficiency of the vegetation as a soil shield against raindrop impact, frost action, and wind is reduced, and decreasing amounts of dead plant material are added to the soil litter horizons. "Invader" and "increaser" species, however, are sufficiently numerous and sufficiently vigorous for maintenance of this thinned plant cover.

This is particularly the case on schist terrain. Continued moderately heavy grazing of modified grassland on schist-derived soils has not led to substantial soil loss and active erosion (Fig. 13), except that due to channelling of run-off along steep and heavily used trails (Fig. 14) or, locally, where

FIG. 12—*Poa cockayniana* association showing the main cover afforded by the lax tussocks of *Poa cockayniana* with the scattered upright toussocks of *D. flavescens* (t.b.l.) and bushes of *Olearia ilicifolia.*
(Plot of column (i), table 2)

FIG. 13—A 35°–40° slope, schist terrain, *D. flavescens* (s.n.l.) association, strongly modified even to the extent of heavy grazing of the *D. flavescens* yet no signs of active accelerate erosion.

slopes are very steep and bed-rock is strongly shattered. On greywacke terrain, however, active accelerated erosion is apparent on all slopes exceeding 20° wherever modification of the sward is far advanced. This leads rapidly to complete soil loss and the spewing of debris into the streams (Fig. 15). The reasons underlying this marked difference in susceptibility to erosion are not known but the effects are obvious.

Apart from this, there is striking soil loss wherever *Danthonia rigida* and *Carpha-Oreobolus* associations are grazed or tracked by animals. The damage here is certainly an effect of trampling. The deep peaty soils supporting these associations are cut into readily by sharp hoofs and are removed rapidly by water. (See Fig. 5.) The total area occupied by these associations is, however, not great, and slopes are gentle. Of greater long-term significance is the effect of grazing and trampling on the grasslands at their maximum altitudinal limits and on the pioneer plant communities developing on the scars left by normal erosion processes. Both habitats are favoured by animals, the short open swards at high altitudes by chamois and hares, and the sparsely covered ground on old erosion sites at lower elevations by deer and chamois. At high altitudes the net effect must be steady though unspectacular whittling back of the vegetation limits and consequent increase in the area of alpine barrens. The effect of grazing country recovering from the operation of normal erosion processes must be to delay recovery. Factual evidence in support of these conclusions is difficult to obtain but in both cases the plants present are normally growing at or near their site tolerance limits. The addition of one more adverse factor, animal use, must have a marked effect. The probability is that some high altitude pockets of grassland have already disappeared without trace. Once the swards are opened, complete removal of all vestiges of soil is very rapid.

The condition, and trends in the condition, of the vegetation on all plots established has been assessed from study of specific frequency and point cover data. The total number of plots is, of course, insufficient for condition and trend data taken therefrom to possess any statistical significance with respect to the Hokitika grasslands as a whole, but the figures do usefully illustrate many of the points at issue. Watershed condition classes recognised were excellent, good, fair, and poor; trend classes were upward, stable, and downward. For the 43 plots established the condition rating was excellent (4), good (20), fair (13), and poor (6). Trend ratings were upward (12), stable (11), and downward (20).

An example of a plot where the condition rating is excellent and the trend stable, is the *Danthonia flavescens* plot of column (c), table 2. Climax or near-climax conditions exist, there is no evidence of any decrease in grazing susceptible species or of increase in resistant species, and soil cover is good. For the *Danthonia rigida* plot, column (a), condition is poor and trend downward. The sward is composed wholly of species resistant to grazing, but owing to the rapidity of soil loss even these are not spreading. Point cover data for this plot reveals 19% of all points as erosion pavement – a very high figure for schist soils in the Hokitika river catchment. The *Poa cockayniana* plot, column (i), is an example of a sward in fair to good condition and with an upward trend. There are small amounts of many really

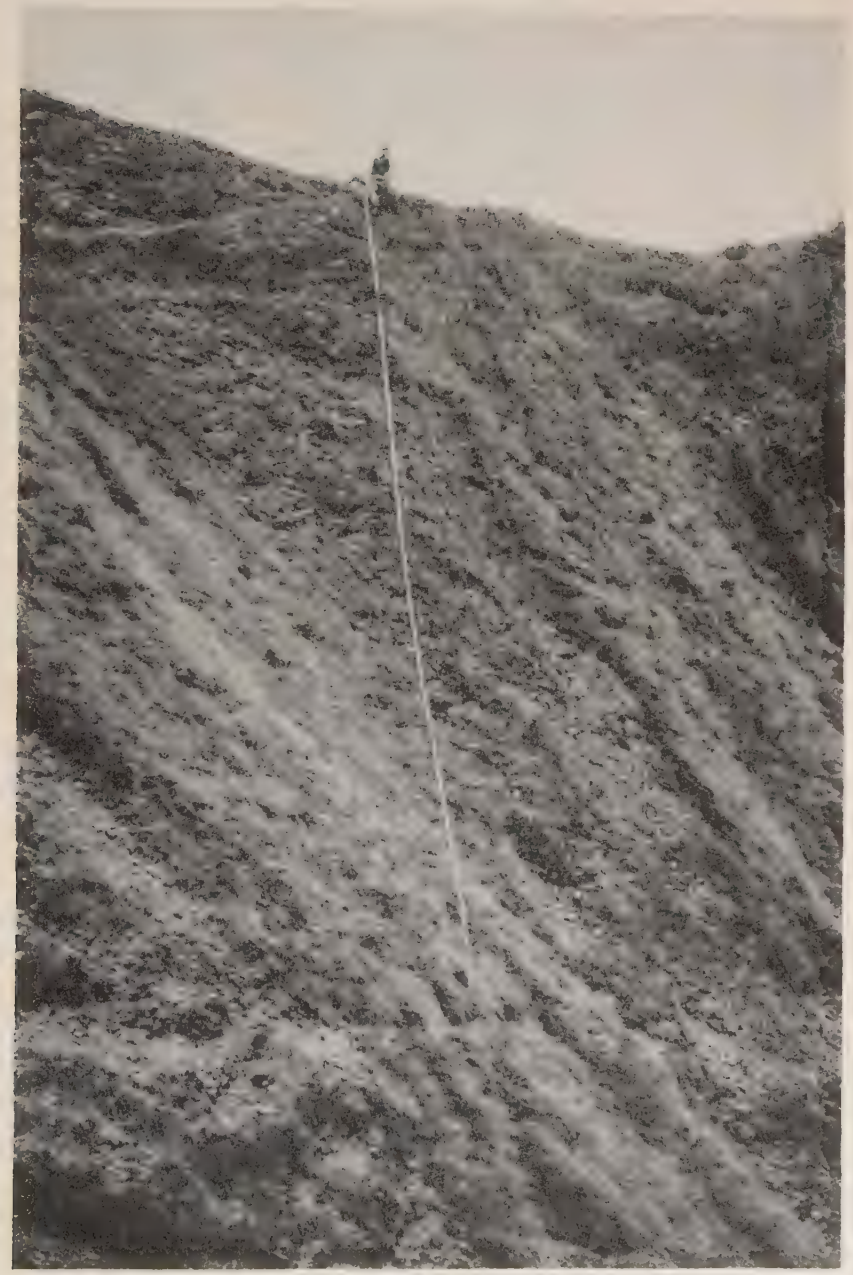

FIG. 14—*D. flavescens* (s.n.l.) association on schist terrain, slope 40° – Zit Saddle, a travelling route used very heavily by deer, chamois, and hares, showing one of the few examples of active accelerated erosion in schist terrain.

grazing susceptible species present and *Danthonia flavescens* is an active invader.

These three examples will serve to illustrate the complexity of the situation. Both condition and trend may vary, even within the one association and at points but a short distance apart. The effect of grazing must frequently depend on small local variations in site and these local variations will also strongly influence the degree of animal use. The degree of use is, moreover, markedly influenced by the extent of sward modification itself. Thus it appears probable that dense near-virgin grassland is not as attractive to deer as lightly modified grassland, the favoured food species being hidden in the rank growth of the less favoured dominants. The grasslands are then systematically grazed outward from the first patches to undergo modification, these pockets being heavily grazed while adjacent areas originally comparable in all respects remain virtually untouched. On greywacke terrain, soil erosion patterns follow closely this irregular grazing pattern.

Trend in condition is also, of course, strongly influenced by local variations in intensity and ease of hunting. Interpretation of long-term trends demands accurate knowledge of the direction and precise location of hunting pressure, coupled with the pinpointing of animal kills. Available records do not provide the required data. For all immediate purposes, however, the over-all situation is clear.

Though the grasslands of the Hokitika river catchment remain, in large part, in fair to excellent order, and though much appears to be currently

FIG. 15—*D. flavescens* (s.n.l.) association on greywacke terrain, slope 25°–30°, showing active accelerated erosion following strong modification of the sward by grazing animals

stable or even to be improving in condition, there is, nevertheless, much grassland in poor order and with a downward trend. This has led, on schist terrain, to the development of a few erosional "sore-spots" and, on greywacke terrain, to acceleration of erosion on a far wider scale. From the viewpoint of watershed management, it is these "sore-spots" to which attention must be directed, even though the greater part of the grasslands may remain in fair condition and capable of sustaining moderate grazing pressure indefinitely.

Finally, no natural reduction in grazing pressure is in sight. Despite extensive modification of the grasslands and reduction or elimination of many favoured, grazing-susceptible plant species, the capacity of the swards to sustain grazing (soil erosion factors apart) remains high. As an indication, plots were rated (poor to excellent) solely according to their condition with respect to grazing. For the 43 plots condition rating from the animal viewpoint was excellent (1), good (14), fair (20), and poor (8). Control of animal populations, other than natural control, is therefore necessary irrespective of any need for control in the interests of other vegetation classes (forest and scrubland) present in the catchment. This control must be exercised, in the first place, on the greywacke lands.

Note on Differences in Composition Between the Alpine Grasslands of the Hokitika River Catchment and the Grasslands of Adjoining Canterbury Catchments

The alpine grasslands of the river catchments to the east of the main divide are far better known than those to the west. The following outstanding points of difference between the two areas are of interest.

1. Indigenous species of frequent occurrence in Canterbury alpine grassland but rare in or absent from the Hokitika grassland:

> *Poa caespitosa, Festuca novae-zelandiae, Danthonia pilosa, D. semiannularis, Leucopogon fraseri, Muehlenbeckia axillaris, Gaultheria depressa, Celmisia spectabilis, Wahlenbergia albomarginata, Helichrysum bellidioides.*

One or two of these, e.g. *H. bellidioides* may, however, occur in quantity in the Hokitika grasslands in restricted areas very close to the main divide.

2. Exotic species common in Canterbury alpine grassland but not found to the west of the main divide:

> *Rumex acetosella, Hypochaeris radicata, Trifolium repens, Agrostis tenuis, Holcus lanatus, Festuca rubra.*

The only exotic species found were *Crepis capillaris* and *Lolium perenne*, the latter being represented by a few small plants established near a site where materials packed in straw had been dropped from the air.

ACKNOWLEDGMENTS

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APPENDIX

Botanical names used in this paper are according to Cheeseman (1925), with the following exceptions:

NAMES USED IN PRESENT PAPER	NAME IN CHEESEMAN
Danthonia rigida Raoul	*Danthonia raoulii* Steud.
Danthonia flavescens Hook. f.	*Danthonia raoulii* var. *flavescens* Hack. ex Cheeseman
Danthonia setifolia (Hook. f.) Cockayne	*Danthonia semiannularis* var. *setifolia* Hook. f.
Poa mackayii Buch.	*Poa kirkii* var. *mackayii* Hack. ex Cheeseman
Petriella colensoi (Hook. f.) Zotov	*Ehrharta colensoi* Hook. f.
Coprosma pseudocuneata Oliver	*Coprosma cuneata* J. D. Hook.
Mecodium demissum (Forst.) Copeland	*Hymenophyllum demissum* Swartz
Meringium multifidum (Forst.) Copeland	*Hymenophyllum multifidum* Swartz

A MODIFIED TEICHERT METHOD FOR THE ESTIMATION OF FAT IN MEAT

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(Received for publication, 12 February 1959)

Summary

A modified Teichert method is described for the rapid estimation of fat in meat and meat products. After mincing the material, the sample is weighed in a weighing beaker attached to the rubber bung of a special 50%, 0·5% subdivided Teichert-type butyrometer.

Digestion is effected with sulphuric acid of s.g. 1·820–25/15°C in a hot water bath maintained at 65°C, and may be aided by mechanical agitation. The results obtained agree within approximately ± 0·5% with those of the Schmid-Bondzynski-Ratzlaff method. For mass analyses serial pipetting units for sulphuric acid and amyl alcohol may be used.

Introduction

The ease of execution of Gerber's acidbutyrometric method for the estimation of fat in milk (British Standards Institute, 1955) led very early to suggestions that this rapid test could be adapted for the estimation of fat in meat products (Kita, 1904), but there is little evidence that these have found much practical application. Peltzer (1936) again drew attention to the possibilities of modified Gerber techniques for meat testing, and Talbot (1949) devised a modification of the Gerber test for fat in sausages and sausage meats. The British worker used standard Gerber milk butyrometers and, in essential details, followed the procedure laid down for dried milk in B.S. 696: Part 2. This offered the advantage that the test could be carried out in every laboratory equipped with Gerber apparatus for milk testing. On the other hand, the modification had the drawback that milk butyrometers are unsuitable for meat testing. With an internal diameter of 11·5 mm the neck is too narrow to permit easy transfer of a minced meat sample; the bulb volume of 1·5 ml is insufficient to reduce the level of liquid in the butyrometer body, which is an essential prerequisite for effective digestion by way of manual or mechanical agitation of the contents. In addition, the graduation of the butyrometer scale, which is designed for an 11·2 g sample, necessitates multiplication of the reading by a relatively large factor (11·2/weight of sample). It has therefore been suggested that the van Gulik cheese butyrometer could be substituted for the milk butyrometer (Pohja *et al.* 1956). Although this butyrometer overcomes some of the disadvantages of the milk butyrometer, it does not permit utilisation of automatic serial pipetting units and renders mechanical agitation hazardous, so that the technique does not recommend itself for mass analyses. It is significant that in other fields, for instance in the analysis of chocolate and cocoa products, modified Gerber techniques have found general application only after special butyrometers, which were better adapted for the materials to be tested, had been designed. With this in view, experiments were made to develop a Teichert type butyrometer for the testing of meat and meat products, and to modify the basic Gerber technique accordingly.

EXPERIMENTAL

Description of Butyrometer

Only minor modifications were necessary to evolve from Teichert's dried milk butyrometer (Gerber *et al.* 1951) the "meat butyrometer" shown in Fig. 1. Butyrometers according to Teichert are manufactured in two models, i.e., direct reading 35% and 70% butyrometers with wide neck and a charge of 2·5 g. The size and volume of their bulb is not specified (Physikalisch

FIG. 1—Meat butyrometer

Technische Bundesanstalt, pers. comm.) and varies considerably with different makes. It was decided to experiment with a specially made series of wide neck, direct reading 50%, 0·5% subdivided "Fucoma" butyrometers, for a 2·5 charge, and a large bulb of 6·5 ml capacity. With an internal diameter of 18 mm the neck was kept wide enough to permit introduction of a perforated 15 mm by 35 mm weighing beaker, which is attached to the butyrometer's rubber stopper by means of a fused on, 23 mm long glass rod, fitting into the centre bore of the stopper. The overall dimensions of the butyrometer are the same as those of standard Gerber milk butyrometers, in order to permit utilisation of normal Gerber equipment.

PROCEDURE

The sample is passed at least three times through a mincer adjusted to the finest possible setting or, alternatively, is thoroughly macerated. If a fat content of less than 50% is anticipated, 2·5 g of the mince are weighed in the previously tared weighing beaker, by placing the beaker with stopper attached directly on the pan of the balance. If the sample is likely to contain more than 50% fat, only 1·25 g are taken. Should the consistency of the mince render weighing difficult, an approximate quantity (2·5 g or 1·25 g) of the sample may be scooped into the weighing beaker, and the exact weight recorded. To the butyrometer is added 10 ml sulphuric acid s.g. 1.820–25/15°C, 7·5 ml cold distilled or soft water, and 1 ml amyl alcohol, complying with the specifications of section eight (British Standards Institute, 1955, Part 2). When a large number of analyses must be made, automatic pipetting units for sulphuric acid and amyl alcohol may be used to facilitate the filling of the butyrometers. The weighed sample in the weighing beaker is introduced into the butyrometer by screwing the stopper firmly into the neck, and the contents are mixed by inverting and shaking the butyrometer. In the case of easily digestible tissues, digestion of the sample is completed by placing the butyrometer into a thermostat controlled Gerber bath maintained at 65°C. If the mince contains tissues which do not break down readily, it may become necessary to transfer the sample from the hot bath to an insulated mechanical shaker as used in the analysis of spray skim milk powder (Falkenhahn 1957). When complete disintegration of the sample has been achieved in the mechanical shaker, the butyrometer is returned to the hot bath for a further period of approximately 5 min. After digestion, the sample is transferred to a preheated, preferably thermostat controlled Gerber centrifuge, and spun for 5 min. at a speed of 1,100 r.p.m. and a temperature of 65°C. The reading is taken at 65°C from the acid/fat interface to the bottom of the upper meniscus. The accuracy of the reading may be improved by transferring the sample from the centrifuge into a Gerber hot air bath, where it is left for 5 min. prior to the reading of the result in a Gerber reading stand. With a 2·5 g sample the reading is direct. When another weight has been taken, the results are calculated by the formula:

$$\frac{2{\cdot}5 \times \text{Reading}}{\text{Weight of sample (in g)}} = \% \text{ fat}$$

Results

The modified method was tried on different meats and meat products, and compared with the two most commonly used gravimetric methods, the Schmid-Bondzynski-Ratzlaff (Schweiz. Ver. Anal. Chem. 1937a) and the Soxhlet (*Ibid.* 1937b) methods.

In the latter, light petroleum ether with a boiling range of 40°–60°C was used as the solvent and extraction was continued for 8 hr. A representative selection of the means of duplicate determinations on raw, cooked, and smoked samples are shown in table 1.

TABLE 1—Comparison of Results Between mod. Teichert and Gravimetric Methods

(Means of duplicate determinations)

Material	Mod. Teichert. % fat	S.B.R. Method % fat	Soxhlet Extr. % fat
Beef sausage	26.0	26.37	25.78
Beef mince	29.5	29.18	28.54
Pork sausage	24.0	24.57	23.63
Pork mince	19.0	18.63	18.02
Liver sausage	18.5	18.82	17.67
Black pudding	12.0	12.23	11.84
White pudding	14.5	15.03	14.12
Ox heart	2.5	2.43	1.93
Calf fry	4.0	3.69	3.18
Kidney	7.5	7.76	6.85
Tripe	1.5	1.73	0.81

DISCUSSION

In common with all volumetric fat tests, the modified Teichert method described has definite limitations. The butyrometer graduation is based on fats of a specific gravity of 0·900. Whereas butterfat will not fluctuate appreciably about this mean (Roeder 1948), the variations in the specific gravity of animal body fats may be wider and within the range of approximately 0·900 ± 0·020 (Hilditch 1947), i.e., a maximum error of ± 0·25% may thus be introduced for a 2·5 g sample and a reading of 25% fat. Since the specific gravity of animal body fat varies, little advantage would have been gained by a correction of the basis of graduation. A further error may be introduced through the reading, because the smallest scale division of 0·5% does not permit estimation of smaller fractions than 0·25%. The utilisation of a weighing beaker eliminates the necessity for transferring the sample, which reduces losses in transfer and the weighing error to negligible proportions. With minced or macerated meats, the difficulty of obtaining a completely homogeneous sample may constitute the most serious source of error which, however, is manifest in volumetric and gravimetric analyses alike. In most instances the difference between Schmid-Bondzynski-Ratzlaff and Teichert results did not exceed ± 0·5%. Greater differences were found occasionally with materials containing tendinous tissues. Such samples are

difficult to mince and it was found impossible to secure fully homogeneous portions. The results obtained by Soxhlet extraction with light petroleum ether were invariably low. Even when extraction was continued beyond 8 hr, a small residue of the fat still remained in the material. The Schmid-Bondzynski-Ratzlaff method was therefore preferred as gravimetric reference method. Under the conditions of the modified Teichert method, during digestion, the formation of interfering carbonised matter reported by Pohja *et al.* (1956) did not occur, or was so slight that it did not affect the reading. Seasoning and spices added to sausages and sausage meats in commercial quantities did not interfere seriously with the reading.

CONCLUSIONS

The agreement obtained between the modified Teichert and the Schmid-Bondzynski-Ratzlaff methods is sufficiently close for most purposes of routine analysis. Owing to the variations in the specific gravity of animal body fats, the limits of error cannot be reduced by changing the basis of graduation of the suggested Teichert meat butyrometer. On the materials tested, the modified Teichert method works satisfactorily and is not appreciably affected by the formation of carbonised matter, or by the interference of seasonings when present in commercial quantities.

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SULPHUR ISOTOPIC VARIATIONS IN NATURE

PART 8—APPLICATION TO SOME BIOGEOCHEMICAL PROBLEMS

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Summary

An attempt is made to use measurements of stable sulphur isotope ratios to explain some biogeochemical problems in nature. Laboratory experiments with bacteria included (a) bacterial sulphate reduction, (b) bacterial chemosynthetic oxidation of sulphur and hydrogen sulphide, (c) bacterial photosynthetic oxidation of sulphide, and (d) an artificial sulphur spring.

Results obtained from these experiments have been used to interpret measurements made on natural environments from which sulphur compounds could be isolated, and the ecology of which had been studied previously. The environments investigated were (i) the Cyrenaican Lakes, North Africa, (ii) the sulphur nodules of Lake Eyre, South Australia, (iii) sulphur from Kona, Masulipatam, east coast of India, (iv) a natural sulphur spring at Waipara, North Canterbury, New Zealand, (v) the "sulphur cycle" in some Australian sewage plants, and (vi) mud and sea water from Milford Sound (a fjord), New Zealand.

Bacterial sulphate reduction and chemosynthetic oxidation of sulphides gave enrichment in the lighter isotope (^{32}S) in laboratory experiments. Preliminary work with other bacterial steps in the cycle did not show any conclusive pattern of isotope fractionation. Measurements of naturally occurring deposits gave conflicting results. In some cases, e.g. Milford Sound, the explanation of the $^{32}S/^{34}S$ ratios was relatively straightforward, in others, no explanation could be offered for the apparently anomalous results.

INTRODUCTION

The present work attempts to apply some results, obtained from controlled laboratory experiments on various steps in the "sulphur-cycle", to explain reactions occurring in the natural environment. The first part deals with experiments on microbiological systems, which are among the most important in controlling biological transformations of sulphur compounds in nature. The second part deals with studies made directly on sulphur compounds in a number of environments where the "sulphur-cycle" takes place.

The early work of Thode *et al.* (1951) showed that the sulphate reducing bacterium, *Desulphovibrio desulphuricans* was capable of preferentially selecting the lighter isotope during reduction of sulphate to sulphide. More recently, Harrison and Thode (1958) have re-confirmed the earlier results and found that the degree of fractionation was proportional primarily to the rate of reduction, but also to sulphate concentration at very low concentrations. These last mentioned results were obtained with resting cell prepara-

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tions. Jones *et al.* (1956, 1957) showed with growing cells of *D. desulphuricans* that rate of reduction, and also concentration of sulphate (at all concentrations) were the controlling factors determining degree of fractionation. In each case, temperature was considered to be important only in so far as it influenced rate.

Jones and Starkey (1957) also attempted to determine fractionation during the oxidation of elementary sulphur to sulphate by the bacterium *Thiobacillus thiooxidans,* but could not detect any.

Use of this property of bacterial fractionation of the sulphur isotopes during reduction of sulphate has been made by Thode *et al.* (1951, 1953, 1954), Feely and Kulp (1957), and Jensen (1958) in explaining a number of phenomena occurring naturally. These works illustrate the usefulness of the isotope techniques in studying certain geological sedimentary processes. They suffer, however, from two defects. The first is that only one step in the cycle viz. (sulphate to sulphide) has been considered as being responsible for fractionation. The second is that, apart from the bacteriological work done by Butlin and Postgate (1954) on the Cyrenaican Lakes, virtually no microbiological work has been done. In those instances, when such work has been attempted, the environment was so complex that results require very cautious treatment.

The present work, although possibly impaired by the exploratory nature of much of the bacterial laboratory work, which is now being repeated in greater detail, is based largely on studies on some relatively simple environments. These environments have been examined biologically either by the present authors or by others.

Since every environment has its own peculiar ecological, chemical, and physical conditions, it is necessary to discuss each separately. As the chemical preparation of the sulphur compounds and the isotopic techniques involved have already been described in detail by Rafter (1957), and Hulston and Shilton (1958) they will not be further discussed here.

LABORATORY EXPERIMENTS

Bacterial Sulphate Reduction

Experiments were done over a wide period of time, using bacterial cultures isolated from various sources, and also different sulphate concentrations. In all cases the work was done with growing cells.

Table 1 illustrates the results obtained with cultures grown in closed bottles. It shows that a definite and significant fractionation occurs in all cases irrespective of the sulphate type used, and also independent of the hydrogen source (whether lactate or steel wool) or source of bacterial inoculation. In all cases the amount of sulphide formed in the above experiments showed that only a small fraction of the sulphate was reduced. The results shown here confirm those of Thode *et al.* (1951), who obtained a maximum fractionation of 1·1‰, and correspond closely with the results for a number of cultures given by Jones and Starkey (1957). In these experiments the cultures were grown at room temperature (18°–28°C) and no attempts were made to measure the rate of reduction. No valid explanation can therefore be given for differences in ‰ enrichment of the lighter isotopes.

TABLE 1—Isotope Fractionation by Sulphate Reducing Bacteria in Separate Experiments

Expt. No.	Lab. No.	Source of Bacteria	Hydrogen Source	$SO_4^=$ Type	$[SO_4^=]$ g/l	$^{32}S/^{34}S$ $SO_4^=$	$^{32}S/^{34}S$ $S^=$	$\delta\,^{34}S$ Enrichment of $S^=$ w.r.t. $SO_4^=$ (‰)	
1a	AB345/8a	Lake Eyre mud	Lactate	Broken Hill Gypsum	Saturated			− 7	
1b	AB345/8b	,,	,,	,,	,,			− 7	
2a	AB345/10	,,	,,	Lab. $CaSO_4$	,,			− 7·5	Gas*
	AB345/10A	,,	,,	,,	,,			−10	FeS†
2b	AB345/10c	,,	,,	,,	,,			−14	Gas*
	AB345/10d	,,	,,	,,	,,			−12	FeS†
	AB345/12	,,	,,	,,	,,			−12	S^0‡
3a	R14/2	Marine	Steel Wool	$Na_2SO_4 \cdot 10\,H_2O$	15·00	22·12	22·36	−11	
3b	R14/3	,,	,,	,,	15·00	22·12	22·34	−10	
4a	R92/10	Lake Eyre mud	,,	,,	15·00	22·13	22·33	− 9	
4b	R91/11	,,	,,	,,	15·00	22·13	22·27	− 6	
4c	R92/12	,,	,,	,,	15·00	22·13	22·36	−10	
5	R152/4	Marine	,,	,,	15·00	22·12	22·36	−10	

* H_2S in gaseous phase and in solution removed by a stream of nitrogen.

† Mass of cells and ferrous sulphide formed through reaction of $S^=$ with Fe^{++} in solution.

‡ Elementary sulphur formed on top of culture medium probably due to a slight leakage of air.

Table 2 represents the results of an experiment using the same inoculum of bacteria (isolated from a marine mud) and grown in a medium containing 5·0 g of 70% w/v, sodium lactate (0·03 mole) at room temperature (22°–25°C). Experiment A5, A6, B5, and B6 were run in 300 ml containers, and C5 and C6 in 500 ml containers. H_2S was continually removed by blowing nitrogen through the flasks and the sulphide captured in 5% $AgNO_3$ solution as Ag_2S. The time intervals in table 2 represent times of removing the silver sulphide and replacing by fresh silver nitrate solution. The chief variable in this experiment is the sulphate concentration, but since a controlled amount of lactate was used and not in great excess, the rate of reduction was also proportional to lactate concentration remaining in the culture vessel in the experiments with higher sulphate concentrations. Thus from the stochiometric equation,

$$SO_4^{=} + 2CH_3CHOHCOO^- \rightarrow S^{=} + 2H_2O + 2CH_3COO^- + 2CO_2 \ldots . (1)$$

it can be seen that 2 moles of lactate are required per mole of sulphate.

From table 2 it can be seen that in experiments A5 and A6, the limiting factor was the sulphate while in experiments C5 and C6, it was the lactate. In B5 and B6 both the sulphate and lactate concentrations probably dropped sufficiently for both to become limiting.

By inspecting table 2 it can be seen that the greatest factor controlling fractionation appears to be the rate of reduction of the sulphate. This can be best observed in A6, B5, B6, C5, and C6. In the first four mentioned there is a sudden decrease in the % enrichment from the first to the second sampling, concomitant with an increase in rate. When the rate drops again towards the end of the reaction the % enrichment again increases as seen in C5 and C6.

It is apparent that concentration of SO_4^- does not appear to play a major role in fractionation from the fact that there is not a large difference between the % enrichment at the different concentration, and also from the fact that the greatest fractionations in C5 and C6 occurred when a maximum bacterial population had been reached and the rate began to decrease rapidly, even though nearly 40% of the SO_4 had been reduced. It may be assumed that concentration does play some role, from the fact that the overall enrichments are greater in C5 and C6 than in the other experiments.

The basic culture media used were similar to those described by Baas Becking and Wood (1955) and by Kaplan (1956). The sulphate reducing bacteria were obtained in "purified" culture by serial transfer. The results therefore cannot be attributed to any particular organisms although *Desulphovibrio desulphuricans* seems most likely.

Bacterial Chemosynthetic Oxidation of Sulphur and Hydrogen Sulphide

A large number of organisms are able to oxidise reduced sulphur compounds in nature. Of these, the bacteria belonging to the genus *Thiobacillus* are amongst the most important. These bacteria are particularly suitable for the present study. In the first place, many species have been isolated in pure culture and can be grown successfully in the laboratory. Secondly, they

TABLE 2—Isotope Enrichment During Sulphate Reduction as a Variant of Sulphate Concentration and Rate

Expt. No.	Lab. No.	$[SO_4^=]$* mg/l at Start of Interval	$[S^=]$ Formed mg/l During Interval	% $SO_4^=$ Reduced During Interval	Interval (hr)	Rate mg S=/hr	$^{32}S/^{34}S$ of S=	$\delta\ ^{34}S$ Enrichment of S= w.r.t. $SO_4^=$ (‰)
A5	R218/34a	300	38.2	38.2	27.5	1.39	22.12	+0.2
	R218/34b		58.6	58.6	5.0	11.7	22.11	+0.6
	R218/34c		3.2	3.2	7.0	0.46	22.07	+2.3
			Total =	100				
A6	R218/35a	300	6.8	6.8	10.0	0.68	22.21	−4.1
	R218/35b		35.2	35.2	17.5	2.01	22.17	−2.1
	R218/35c		51.5	51.5	5.0	10.30	22.09	+1.6
	R218/35d		6.5	6.5	7.0	0.93	22.12	−0.1
			Total =	100				
B5	R218/36a	600	11.2	5.6	10.0	1.12	22.24	−5.3
	R218/36b		44.4	22.2	17.5	2.54	22.16	−2.1
	R218/36c		82.6	41.3	5.0	16.52	22.14	−0.8
	R218/36d		17.0	8.5	7.0	2.43	22.16	−1.7
	R218/36e		2.6	1.3	12.0	0.22	(Too small)	
			Total =	78.9				
B6	R218/37a	600	12.4	6.2	10.0	1.24	22.25	−5.8
	R218/37b		54.4	27.2	17.5	3.10	22.14	−1.1
	R218/37c		77.6	38.8	5.0	15.50	22.11	+0.5
	R218/37d		14.2	7.1	7.0	2.03	22.16	−1.6
	R218/37e		3.4	1.7	12.0	0.28	22.15	−1.4
			Total =	81.0				
C5	R218/38a	1500	8.4	1.7	10.0	0.84	22.23	−5.0
	R218/38b		39.8	8.0	17.5	2.27	22.22	−4.4
	R218/38c		67.0	13.4	5.0	13.40	22.20	−3.8
	R218/38d		48.0	9.6	7.0	6.85	22.20	−4.7
	R218/38e		13.2	2.6	12.0	1.10	22.25	−5.7
	R218/38f		5.4	1.1	5.0	1.08	22.24	−6.0
	R218/38g		18.0	3.6	4.0	4.50	22.28	−7.4
	R218/38h		4.8	1.0	18.0	0.27	22.29	−7.8
			Total =	41.0				
C6	R218/39a	1500	4.0	0.8	10.0	0.40	Lost Sample	.....
	R218/39b		19.8	4.0	17.5	1.13	22.24	−5.2
	R218/39c		55.6	11.1	5.0	11.10	22.24	−5.2
	R218/39d		63.2	12.6	7.0	9.00	22.25	−5.6
	R218/39e		38.0	7.6	12.0	3.13	22.25	−5.6
	R218/39f		9.6	2.0	5.0	1.92	22.27	−6.8
	R218/39g		8.1	1.6	4.0	2.02	22.29	−7.5
	R218/39h		4.8	1.0	18.0	0.27	22.25	−5.9
			Total =	40.7				

*The $^{32}S/^{34}S$ ratio for the sulphate used was 22.12.

oxidise most reduced forms of inorganic sulphur compounds to higher oxidation states, usually sulphate. Thirdly, they are ubiquitous and play an important role in a large number of environments in nature. The physiology and metabolism of these organisms has been reviewed recently by Vishniac and Santer (1957).

In studying the bacterium *Thiobacillus thiooxidans*, Jones and Starkey (1957) were unable to detect any fractionation when it was grown on elementary sulphur. In the present work, the bacterium employed was *T. concretivorus*, obtained in pure culture by Parker (1947), which is similar in most respects to *T. thiooxidans*. Growth experiments were studied using commercial sulphur and sulphur of volcanic origin (from White Island, New Zealand) as the substrate for oxidation. The sulphate produced by the organism was separated and analysed. Enrichment of ^{34}S varied from + 1·6 to — 0·5 mil.

In these experiments the sulphur was always kept in excess as a solid "insoluble" phase in the medium. Aerobic experiments were carried out in Erlenmeyer flasks generally 1/5 full of medium, with the sulphate crystals floating on top.

In preliminary experiments, with *T. concretivorus*, grown on H_2S as the sulphur source, enrichment of the lighter isotope could be detected when the products of the oxidation, sulphur and sulphate, were analysed. Cultures of *T. concretivorus* were grown by C. D. Parker (Melbourne and Metropolitan Board of Works, Australia), in an atmosphere containing approximately 200 ppm H_2S, at room temperature (20°–25°C). After 8 to 10 days the sulphur which had formed as a pellicle was filtered from the culture medium and the sulphate formed in the culture was precipitated as barium sulphate.

TABLE 3—Isotope Fractionation During the Oxidation of H_2S by *Thiobacillus concretivorus*

Expt.	Lab. No.	Sample	$^{32}S/^{34}S$	$\delta\,^{34}S$ enrichment w.r.t. $S^=$ (‰)
1	R13	Sulphide used	22.15	0.0
	R12	Bacterial sulphur	22.22	−3
	R12a	Bacterial sulphate	22.33	−8
2	R218/80a	Sulphide used	22.18	0.0
	R218/80b	Bacterial sulphur	22.32	−6
	R18/80c	Bacterial sulphate	22.37	−8.5
3	R218/80a	Sulphide used	22.18	0.0
	R218/80f	Bacterial sulphur	22.29	−5
	R218/80e	Bacterial sulphate	22.31	−6

The results represented in table 3 demonstrate that there is a significant enrichment of ^{32}S both in sulphur and sulphate. In experiment 3 the sulphate (Lab. No. R218/80e) was not precipitated in the culture solution but was separated by washing the filter papers on which the sulphur had been filtered out, and then precipitating this solution with barium chloride. This may be the reason for the reduced enrichment.

The results of this study therefore indicate that during the oxidation of $H_2S \rightarrow S^0 \rightarrow SO_4^=$ by *Thiobacillus,* the lighter isotope (^{32}S) is preferentially selected. The oxidation of elementary sulphur to sulphate by this genus does not clearly indicate such a selection. If anything, it appears that during such an oxidation the heavier isotope may be selected (Kaplan and Rafter, 1958). Further work is being continued to elucidate this point.

Bacterial Photosynthetic Oxidation of Sulphide

Whereas the *Thiobacilli* are chemosynthetic and can tolerate highly acidic conditions, the photosynthetic sulphur bacteria oxidise reduced sulphur compounds under neutral or alkaline conditions. Their environment differs greatly from that of the chemosynthetic sulphur bacteria in that they require light and strict anaerobic, usually highly reducing, conditions for growth. They are similar, however, in that they are able to oxidise reduced sulphur compounds to sulphate. Since part of their energy requirements is supplied by light, they appear to be able to carry out the oxidation process extremely efficiently under optimum conditions.

In the present study a halophilic purple sulphur bacterium *Chromatium* sp. was used. This organism, when grown on sulphide, is able to store the elementary sulphur formed within the cell, and then further oxidise it to sulphate (van Niel, 1931).

Difficulty was experienced with this work from the beginning. In the first place it was difficult to control growth and bacterial metabolism. Once the bacteria began to grow, which could be judged by the colour of the culture, the sulphide was metabolised at a very rapid rate and converted to sulphate. In most experiments, therefore, it was impossible to compare the effects of rate and substrate concentration. To this complication was added the fact that in a dilute alkaline solution, and especially when exposed to photoactivity, sulphides tend to form polysulphides and polythionates, even under strict reducing conditions. Therefore the results in table 4 are given to indicate the apparent trends. Their true interpretation may have to wait until these experiments have been repeated more critically.

It does appear from table 4 that the oxidation of sulphide to sulphur and sulphate leads to an enrichment of ^{34}S. This enrichment is more apparent in the sulphate. Of the three analyses made on sulphur, one (R218/12) showed a slight enrichment of the ^{32}S. This suggests that the oxidation of sulphide to intracellular sulphur probably occurs without any significant fractionation. The sulphur is then further oxidised to sulphate, in which process the heavier isotope may be preferentially selected. Since this would imply that the sulphur left in the cell should be enriched in ^{32}S, it may also be argued that the initial oxidation of sulphide is a process which selects the heavier isotope, while the further oxidation to sulphate is unselective. A third alternative, of course, is that the equilibrium lies towards formation of $^{34}SO_4^=$ during the entire oxidation.

TABLE 4—Isotope Fractionation by Photosynthetic Sulphur Bacteria Utilising Sulphide as an Energy Source

Expt. No.	Lab. No.	$^{32}S/^{34}S$ of $S^=$	$^{32}S/^{34}S$ of S^o	$^{32}S/^{34}S$ of $SO_4^=$	$\delta\ ^{34}S$ enrichment of ^{34}S in S^o w.r.t. $S^=$ (‰)	$\delta\ ^{34}S$ enrichment of ^{34}S in $SO_4^=$ w.r.t. $S^=$ (‰)
1	AB1478/3f	22.06	–	21.95	–	+4
	AB1478/3g	22.06	–	22.04	–	+1
2	R218/9a	22.24	22.21	22.20	+1.5	+2.0
3	R218/11e	22.24	22.23	22.20	+0.5	+2.0
4	R218/12	22.24	22.27	22.17	−1.5	+3.0

One experiment was done using 10 g/l of sodium thiosulphate ($Na_2S_2O_3$, $5H_2O$) as the reduced sulphur source. Growth, once it started, was very rapid again here, quickly depleting the $S_2O_3^=$. The results, even under these unsatisfactory conditions, showed a fractionation of greater than 3·2 ‰ in favour of ^{34}S.

The experiments 2, 3, and 4 in table 4 were so designed as to allow for observations on the ability of these photosynthetic organisms to fractionate the carbon isotopes ^{12}C and ^{13}C. The organisms were grown in sealed vessels containing 3·0 g/l $NaHCO_3$ as the sole carbon source. After completion of growth, the bacteria were washed and analysed for CO_2 by combustion. A sample of the medium was acidified and the CO_2 remaining was removed and analysed.

Table 5 gives the results observed. The mass spectrometer results were calculated relative to the New Zealand standard, using Te Kuiti limestone (Craig, 1957) and are given as δ values.

$$\delta\ ^{13}C = \frac{^{13}C/^{12}C \text{ sample} - ^{13}C/^{12}C \text{ standard}}{^{13}C/^{12}C \text{ standard}} \times 1{,}000 = ‰ \text{ enrichment } ^{13}C$$

according to the proposal of Craig (1953) and the present usage.

TABLE 5—Fractionation of Carbon Isotopes by Photosynthetic Sulphur Bacteria

Expt. No.	Lab. No.	$\delta\ ^{13}C^*$ in HCO_3^- remaining (‰)	$\delta\ ^{13}C^*$ in Cells (‰)	$\delta\ ^{13}C$ in Cells w.r.t. HCO_3^- remaining (‰)
2	R218/10	+10	−18	−28
3	R218/11	+6.5	−21.5	−28
4	R218/12	+4.5	−21.0	−25.5

*Fractionation given with respect to the original HCO_3^- used as a carbon source.

The results show clearly that in every instance the cells incorporated the lighter isotope to about the same extent. The unused HCO_3^- remaining in solution showed a corresponding enrichment in the ^{13}C. These results agree well with observations made in nature on algae, plankton, and other plant material from analyses given by Craig (1953), Silverman and Epstein (1958).

Artificial Sulphur Spring

In order to reproduce conditions which may exist in nature, an experiment was begun with an "artificial sulphur spring", as sketched in Fig. 1. It consists of a 20 litre reservoir (R) containing sea water fortified with 0·2% NH_4Cl and 0·2% KH_2PO_4. A 2½ litre bottle (M) with an opening at the bottom, was partly filled with mud and two members of the estuarine green algae, *Zostera* and *Enteromorpha*, with a little steel wool to provide strong reducing conditions. The sea water in R was allowed to perfuse through M at a rate of 10 litres/day. It emerged at C, flowed down the slide S and into the gutter G.

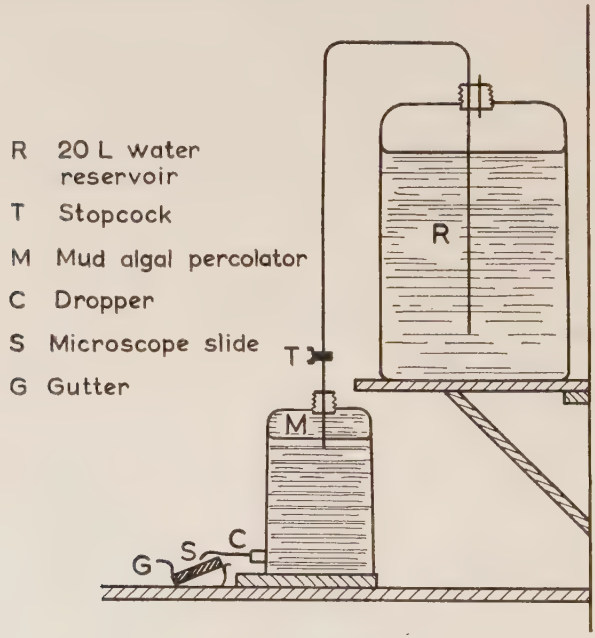


FIG. 1—Artificial sulphur spring apparatus.

The algal mass in M provided sufficient organic nutrients for active sulphate reduction to occur with the liberation of a high content of H_2S. This was present in solution in the effluent water at C. While flowing over the microscope slide bacterial oxidation took place, resulting in the formation of a film of elementary sulphur impregnated with masses of *Thiobacillus* and Beggiatoaceae. This has been described in greater detail by Baas Becking, Wood, and Kaplan (1956).

A sample of sea-water solution similar to that used in R was stored for some weeks in a sealed glass bottle. A slight amount of sulphate reduction (1% of $SO_4^=$ in solution) occurred yielding sufficient sulphide for an analysis. This is given as Lab. Sample No. R 92/9b, Table 6, and shows an extremely high enrichment of ^{32}S. This is probably due to a very slow rate of reduction due to lack of a sufficiently large source of hydrogen donor.

TABLE 6—Fractionation of the Sulphur Isotopes in the Sulphate of Sea Water in an Artificial Sulphur Spring Experiment

Lab. No.	Sample Analysed	$^{32}S/^{34}S$	$\delta\ ^{34}S$ enrichment w.r.t. R92/9a (‰)
R92/9a	$SO_4^=$ in R*	21.72	0.0
R92/9b	$S^=$ in R†	22.45	−32.5
R92/7	S° in S	22.34	−28.2
R92/8a	$S^=$ in G	22.20	−21.4
R92/8b	$SO^=_4$ in G	22.09	−16.8

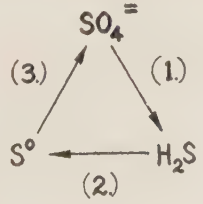
*The letters refer to Fig. 1.
†$S^=$ developed in a sealed vessel containing a sample of sea water solution used in R.

The effluent water was collected in the gutter after flowing down the slide and used for analysis for $S^=$ and $SO_4^=$. These samples are represented by Lab. Nos. R92/8a and R92/8b respectively. The elementary sulphur on the slide is represented by Lab. No. R92/7.

From table 6 it can be seen that under optimum conditions a fractionation of over 30 ‰ can be attained. The results given by the $S^=$ in R92/8a show that here a fractionation of over 20 ‰ was attained. It may well be, however, that originally this fractionation was greater since the sulphur, R92/7 shows a fractionation of 28 ‰ and was formed through the oxidation of $S^=$, R92/8a. If during this oxidation, the ^{32}S was selectively oxidised, it would account for the observations. The sulphate, R92/8b, is also enriched in ^{32}S with respect to the sulphate in sea water. It is possible that this may have resulted from the addition of sulphate enriched in ^{32}S by the oxidation of elementary sulphur and H_2S by sulphur bacteria.

Discussion

The experiments discussed in the preceding pages involve three important steps in the "sulphur cycle" depicted below



The results of the isotope studies are summarised in table 7. It can be seen that while the sulphate reduction (1) step produces an undisputed fractionation, this has not been demonstrated so well in the other steps. It does appear, however, that enrichment of the lighter isotope takes place during oxidation of H_2S to S^0 and $SO_4^=$, (2) and (3), by *Thiobacilli*. Formation of $SO_4^=$ from H_2S by the photosynthetic sulphur bacteria, and from elementary sulphur by *Thiobacilli* may lead to an enrichment of ^{34}S.

TABLE 7—Summary of Bacterial Processes Involved in the Cycling and Fractionation of Sulphur

Process	Micro-organisms involved	Fractionation
1. $SO_4^= \rightarrow H_2S$	Sulphate reducing bacteria *Desulphovibrio desulphuricans*	Pronounced enrichment of ^{32}S, 32·5 $^0/_{00}$ measured.
2. $H_2S \rightarrow S^0$	Sulphur oxidising bacteria	Enrichment of ^{32}S.
	Chemosynthetic + *Thiobacillus* + Beggiatoaceae	
	Photosynthetic + Thiorhodaceae	Doubtful.
3. $S^0 \rightarrow SO_4^=$	Sulphur oxidising bacteria *Thiobacillus concretivorus*	Enrichment of ^{32}S when grown on H_2S. Possible slight enrichment of ^{34}S when grown on S^0.
	Thiorhodaceae *Chromatium*	Possible enrichment of ^{34}S.

NATURAL DEPOSITS

General

A series of natural environments, in which the "sulphur cycle" plays a role, have been investigated. In many of these, microbiological studies have been carried out either by the present authors or by others. The descriptions which follow will largely be aimed at elucidating a number of steps and critically discussing, from a biological point of view, previous work on similar environments.

In the first place a summary will be given of the known processes occurring. The observed isotope measurements will then be discussed in the light of present knowledge. It may be possible to use certain data from natural deposits to aid interpretation of laboratory experiments.

MICROBIOLOGICAL SULPHUR FROM CYRENAICAN LAKES, NORTH AFRICA

The results of isotope measurements originally made by MacNamara and Thode (1951) have been widely quoted in the literature and taken as an example of a maximum known fractionation observed in nature as a result of sulphate reduction. The measurements were recently repeated by Harrison and Thode (1958) on other samples, and have also been made during the course of the present work. The results are given in table 8.

In this work a sample of crude sulphur was obtained from Dr K. R. Butlin in 1956. Analysis of this sulphur gave $S^0 = 51{\cdot}4\%$, $SO_4^= = 5{\cdot}2\%$,

(this sulphate being used for analysis). It is unfortunate that Thode and co-workers do not indicate the source of the sulphate analysed, since it is undoubtedly of importance in any interpretation.

The microbiological formation of sulphur in the Cyrenaican Lakes has been fully discussed by Butlin and Postgate (1954). It appears to take place through the influence of two major bacterial processes. The first two involve the reduction by *D. desulphuricans* of sulphate, introduced by a warm water spring. The H_2S produced is then oxidised mainly by the activity of the purple and green sulphur bacteria – *Chromatium* sp. and *Chlorobium* sp. – and possibly to a small extent by atmospheric oxygen. *Thiobacilli* could not be isolated from water and mud samples, and probably only played a small role (presumably because of the highly reducing conditions existing throughout).

From the analysis quoted by Butlin and Postgate (1954) for one of the lakes, Ain-ez-Zauia, it is possible to calculate approximately the percentage of sulphate being reduced by bacterial activity in the bottom waters

$$\text{Concentration of sulphur as } S^{=} \simeq 0{\cdot}01\%$$
$$\text{Concentration of sulphur as } SO_4^{=} \simeq 0{\cdot}06\%$$

Thus the ratio, *sulphur as sulphide* : *sulphur as sulphate* $\simeq$ 1 : 6. This indicates that a high proportion of the soluble sulphate is being reduced, and probably means a rapid rate of reduction since the H_2S is continually being removed and metabolised. If this assumption is correct, it would appear doubtful that as high a fractionation as that obtained by MacNamara and Thode (1951) could be obtained by the process of sulphate reduction alone. As has already been described in the section on photosynthetic bacteria, these organisms are capable of oxidising sulphide to sulphate. That this oxidation probably occurs, can be judged from the analysis of the sample of sulphur, quoted above, in which the $SO_4^{=}$ content is 5·2%.

Thus, sulphate formed again by photosynthetic bacteria, with only a slight enrichment of ^{34}S compared to that in the sulphide, will be continually available for further reduction and fractionation by *D. desulphuricans*. This could yield a sulphide greatly enriched in ^{32}S with respect to the original sulphate issuing from the warm water spring, depending on the degree of intermixing of $SO_4^{=}$ ions and the number of cycles involved.

The above reasoning may well account for the differences in absolute isotope ratios and in fractionation obtained from different samples given in table 8. The values, therefore, do not necessarily signify fractionation by a single bacterial process.

TABLE 8—Isotope Fractionation in Cyrenaican Lakes

$^{32}S/^{34}S$ of $SO_4^{=}$	$^{32}S/^{34}S$ of S°	$\delta\,^{34}S$ enrichment (‰)	Reference
21.88	22.57	−31	MacNamara & Thode (1951).
22.190	22.518	−14.6	Harrison and Thode (1958).
22.16	22.56	−18	This work (Lab. No. R92/14).

MICROBIOLOGICAL SULPHUR NODULES OF LAKE EYRE, SOUTH AUSTRALIA

The sulphur nodules of Lake Eyre have been described in detail by Bonython and King (1956) and by Baas Becking and Kaplan (1956). The latter authors, after a study of the ecological conditions existing, postulated the process to occur according to Fig. 2.

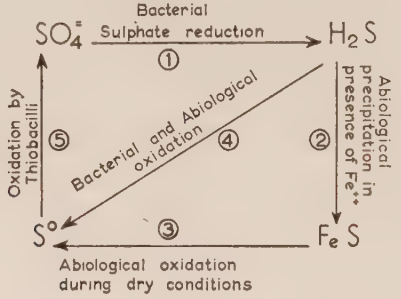


FIG. 2—The cycle suggested to be responsible for the sulphur nodules at Lake Eyre.

Since at least three different forms of sulphate are recognisable, in addition to the elementary sulphur, it was considered of interest to determine whether these can be distinguished isotopically, and whether the isotopic results can be explained by the biological observations. The results are given in table 9.

The deposit consisted of mostly spherically shaped nodules, a few inches in diameter, surrounded by a shell of gypsum, the inner part of which appeared to be intimately growing out of the sulphur. It covered an area of about 100 sq. yd. to a depth of 18 inches from the surface, mainly on the west side of Sulphur Peninsula, which is aligned roughly N.N.E.–S.S.W., on the southern shore of Lake Eyre. The deposit on the east side was much more confined and smaller, appearing to have been re-worked. Sulphur Peninsula was covered with gypseous sand, which formed cliffs outlining the ancient shore line.

It can be considered that the cliffs constitute the original sedimentary material which supplied $SO_4^=$ for sulphate reduction and sulphur formation. The isotope ratio of AB 345/6, Table 9, compares favourably with the ratio measured for a large gypsum crystal of sedimentary origin from Broken Hill $^{32}S/^{34}S = 22{\cdot}11$. All fractionations in table 9 are therefore given relative to sample AB 345/6.

Sample R/7 is the sulphate isolated from the black mud beneath a thin salt crust near the northern tip of Sulphur Peninsula. This mud was black and highly reducing. The isotope ratio represents an enrichment of 8 $‰$ in the ^{34}S which would be expected from the knowledge that sulphate reduction was in progress in the mud. The sulphur nodules themselves show a high enrichment of ^{32}S. Sample AB 345/2b is enriched by 20 $‰$ with respect to the gypsum in the cliffs and 28 $‰$ with respect to the $SO_4^=$ in the mud from which it must have formed. The gypsum in contact with the sulphur is like-

wise enriched in ^{32}S with respect to the sulphate in the mud and in the cliffs. Thus samples AB 345/1 and AB 345/2a are enriched by 27 ‰ and 22·4 ‰ respectively with respect to the $SO_4^=$ in the mud and the cliffs. These two samples are therefore obviously different from the sulphate in either the gypsum cliffs, or in the mud. The original explanation that they were formed through the oxidation of sulphur by *Thiobacilli* (Baas Becking and Kaplan, 1956) appears to be substantiated.

TABLE 9—Isotope Fractionation of Sulphur and Sulphate at Lake Eyre

Lab. No.	Sample	Locality	$\delta\ ^{34}S$ enrichment* w.r.t. AB345/6 (‰)
AB345/6	Gypsum	Cliffs lining lake edge, Sulphur Peninsula	0.0
R/7	$SO_4^=$	In mud under salt crust near northern tip of Sulphur Peninsula	+ 8
AB345/1	Gypsum crystals around nodules	W. side Sulphur Peninsula	−19
AB345/2a	$SO_4^=$ in sulphur nodules	„	−14.4
AB345/2b	S° from nodule	„	−20.3
AB345/4	Large single gypsum crystal	„	− 5.5
AB345/3	Gypsum crystals on outside of nodule	E. side of Sulphur Peninsula	− 9.1
AB345/5a	Gypsum crystals in contact with sulphur	„	−18.7
AB345/5c	Sulphur	„	−13.3

*All enrichments are given relative to sample AB345/6.

A fourth type of gypsum present on the west side of Sulphur Peninsula is in the form of clear oval crystals about 1 inch long and ½ inch wide. One such crystal, AB 345/4 gave $^{32}S/^{34}S$ ratio higher than from the gypsum in the cliffs, but lower than the secondary gypsum around the sulphur nodules. It is probable that these represent sedimentary crystals precipitating out of solution when the lake was saturated with salts.

MICROBIOLOGICAL SULPHUR AT KONA (MASULIPATAM), EAST COAST OF INDIA

An interesting deposit of sulphur was discovered in 1943 along the coastal regions of eastern India, in the neighbourhood of the village Kona near Masulipatam, Madras. It consists of colloidal and crystalline sulphur associated with clays, silts and sands, generally to a depth of 6 inches or at some places 12 inches from the surface. Immediately below the sulphur-bearing clay is a black moist clay with a high H_2S content, while overlying the sulphur zone is yellow and red oxidised earth. These deposits are lenticular and die out within short distances.

The formation has been described by Iya and Screenivasaya (1944, 1945), and by Subba Rao, Iya, and Screenivasay (1947) as being caused by a bacterial sulphate reduction at depth, followed by formation of ferrous sulphide and atmospheric oxidation near the surface. It appears that certain coastal areas on the Bay of Bengal are liable to be flooded by sea water for over six months of the year. The continual introduction of sulphate, together with the organic matter in the soil, provide an adequate environment for intense activity by sulphate reducing bacteria in the water logged, anaerobic clays. The steps of sulphur formation appear to be identical with steps (1), (2), and (3) of Fig. 2, outlined in the section on the sulphur of Lake Eyre.

The above mentioned authors (loc. cit.) were able to isolate a halophilic spore-forming sulphate reducer in pure culture (probably *Clostridium nigrificans*) and demonstrated the presence of a large number of aerobic bacteria in the sulphur zone.

Two samples of the sulphur-bearing clay were obtained from independent sources. The first was from Dr C. Karunakaran, Geological Survey of India, Calcutta, and the second from Dr J. V. Bhat, Indian Institute of Science, Bengalore. The previous work was carried out in the latter Institute, so it is possible that the sample may have been investigated microbiologically.

Although no macroscopic gypsum crystals are present in the sulphur deposits (Dr Karunakaran, pers. comm.) the present authors were able to extract sufficient $SO_4^=$ for isotope analysis. The results represented in table 10 indicate that sample 1, having the high sulphur content, was probably collected near the surface and in the oxidising zone. Sample 2 probably came from the transition zone, close to the interface of the oxidising and reducing environments and some distance from the surface.

TABLE 10—Isotope Fractionation in Kona Sulphur

Sample No.	Lab. No.	% S°	% $SO_4^=$	$^{32}S/^{34}S$ of S°	$^{32}S/^{34}S$ of $SO_4^=$	$\delta\,^{34}S$ enrichment of S° w.r.t. $SO_4^=$ (‰)
1	R152/6	28.0	7.8	21.70	21.68	−1
2	R218/41	2.4	8.75	21.69	21.68	−0.4

The results are of interest in that no enrichment of the lighter isotope is present in the sulphur from either sample. The sulphur from both localities also shows a constant ratio, indicating that the mode of origin is probably the same. The sulphates also show no variation in their ratios. These sulphates are slightly enriched in ^{34}S with respect to average sea water sulphate (21·76) (Ault 1959) and may indicate a selection through sulphate reduction in the region. It would appear that this sulphate may be a mixture of the original sea-water sulphate together with secondary sulphate formed through the oxidation of hydrogen sulphide and perhaps sulphur.

The anomalously low ratio obtained for the sulphur samples is very puzzling, and it is difficult to formulate any hypothesis at the present stage of knowledge on isotope fractionation in nature.

A NATURAL SULPHUR SPRING AT WAIPARA, NORTH CANTERBURY, NEW ZEALAND

This natural sulphur spring is a true counterpart of the artificial spring constructed in the laboratory and described above. In this environment there existed a sulphuretum (Baas Becking 1925) displaying many of the significant processes in the "sulphur-cycle".

The samples were collected from a very small stream about 30 ft long, 2 ft wide and 8 in. deep. It flowed from the base of a 25 ft Miocene cliff of rudaceous arenaceous conglomerate (which formed the right bank of the Waipara River) into the Waipara River. This Miocene cliff, composed of unconsolidated basal conglomerate, contained many gypsum crystals and yellow silt which had been previously identified as sulphur. Analysis showed that no elementary sulphur was present, but that the yellow substance was probably limonite. This bed was overlain by Tertiary lignite deposits and underlain by Cretaceous glauconite, indicating its origin to be a neritic or littoral environment, in probably a warm, dry, and highly oxidising climate.

The stream coming from the cliff was clear near the base, but about half way down it became covered with a red and yellow scum. Beneath this scum was a black mud with a high content of H_2S (bubbles forming when the mud was disturbed). At its mouth, where it flowed into the main stream, the surface became clear again, but on the bottom were black gelatinous filaments tipped with yellow. The stream appeared to be hardly flowing and may only have been a semi-stagnant arm of the main river flowing past the base of the Miocene cliff.

A microscopic study revealed that the black mud contained *Desulphovibrio*. The red and yellow scum on the surface of the water contained sheathed iron bacteria, *Leptothrix* and *Sphaerotilus*. The black gelatinous material with the yellow was a mass of filamentous sulphur oxidising bacteria belonging to the Beggiatoaceae.

From the field and laboratory observations, a series of steps were postulated to explain the environment. It was considered that the gypsum in the basal conglomerate was dissolved out by the stream and formed its sulphate component. As a considerable amount of the weeds and grass grew beside the stream, these provided the organic matter of the mud necessary to allow

sulphate reduction to proceed. The liberated H_2S reduced the limonite silt and sand, forming a high content of ferrous ion. This was then oxidised at the surface of the stream to ferric hydroxide with the aid of the sheathed iron bacteria. Near the junction of the stream with the Waipara River, turbulent conditions prevailed, and this allowed a mixing of the reducing and oxygenated waters. This allowed the growth of gradient organisms such as the filamentous sulphur bacteria, which catalyse the formation of elementary sulphur.

Table 11 shows that the basal sulphate has a lower $^{32}S/^{34}S$ ratio than the other sulphur constituents. The sulphides, both volatile and fixed, have the highest ratios, with the elementary sulphur lying close to the acid volatile sulphide. The disulphide appears to be the most stable sulphide, since it has the least variation. The high ratio for the sulphate in solution and in the mud is puzzling, but may indicate a rapid oxidation of the H_2S and S^0 to $SO_4^=$ and a mixing with the sulphate derived from the basal conglomerate in the cliff.

TABLE 11—Isotope Fractionation in a Sulphuretum Stream at Waipara

Lab. No.	Sample description	Sulphur compound	% by weight or volume	$^{32}S/^{34}S$	$\delta\,^{34}S$ enrichment* (‰)
R218/17b	Miocene basal conglomerate	$SO_4^=$	1.00	22.23	0.00
R218/15a	Mud from stream	$S^=$	0.088	22.64	−18.5
R218/15b	„	$SO_4^=$	0.025	22.34	− 5.1
R218/15d	„	Non-extractable sulphur†	0.018	22.62	−17.7
R218/16a	Surface water above mud	$SO_4^=$	0.033	22.38	− 6.7
R218/21a	Black and yellow gelatinous mass at junction of stream and Waipara River	$S^=$	0.072	22.51	−12.6
R218/21b	„	S^0	0.040	22.48	−11.3
R218/21d	„	Non-extractable sulphur†	0.018	22.57	−15.5

*All enrichments given with respect to sample R218/17b.

†Analysed by combustion after removal of acid soluble sulphides and sulphates, and after extraction with benzene. Probably represents iron disulphide (pyrite and marcasite) and non-extractable organic sulphur.

THE "SULPHUR-CYCLE" IN SOME AUSTRALIAN SEWAGE PLANTS

The sewage in the vicinity of Melbourne and Sydney was investigated to establish whether any fractionation was occurring. A comprehensive literature exists on the role of microbial activity in sewage, and attention is drawn to the articles by Parker (1951) and Parker and Prisk (1953) as an example of such work concerning the "sulphur cycle".

Of particular interest in this study, is the knowledge that the activities of the acid-tolerant sulphur bacteria are unmistakably pronounced. Through an aerobic oxidation of the free H_2S *Thiobacillus concretivorus* (first isolated in this environment by Parker 1947) is able to produce sufficient sulphuric acid to attack the concrete walls and roofs of tanks and drains, and form as much as 30% gypsum. Often elementary sulphur is mixed up with the gypsum.

TABLE 12—Isotope Fractionation in Melbourne and Sydney Sewage Plants

Lab. No.	Description of Sample	$^{32}S/^{34}S$
	MELBOURNE SEWAGE	
R8	Sulphate from sewage filtrate	22.0
R10	Sulphate from concrete wall of drain	22.2
R10A	Sulphur mixed with sulphate in R10	22.3
R9	Sulphide from sludge evolved with acid and nitrogen	21.9
	SYDNEY SEWAGE	
R92/6	Sulphate from sewage filtrate	22.16
R92/5	Sulphide from sewage (R92/6) evolved with acid and nitrogen	22.37
R92/3	Sulphate from concrete wall of settling tank	22.18
R92/2	Sulphate from water dripping from roof of settling tank (pH 1.5)	22.14
R92/1	Sulphate from acid water (pH 2.0) on floor of sewage plant	22.11
R92/4	Sulphur, by oxidising H_2S in atmosphere of sewage plant	22.19

In table 12, samples R8, R10, and R10A come from a sewage drain near Spotwood while R9 comes from a sewage sludge digestion tank at Braeside, Victoria. Samples R92/1, 2, 3, 5, and 6 come from the sewage plant at Bondi, while R92/4 came from the northern Outfall Sewer, New South Wales. In the last named sewers, the origins of the actual sewage are mainly domestic, and very similar in composition to one another.

A number of puzzling features are present in the results of table 12. The low $^{32}S/^{34}S$ ratio in R9 is difficult to explain on the basis of sulphate reduction. It may be due to a preferential removal of ^{32}S leaving the heavier isotope behind. Even if the H_2S came from the proteolytic decomposition of organic matter, one would expect a higher ratio on the basis of the investigations by Szabo *et al.* (1950), on organic sulphur. The values of R92/6 and R92/5 seem to be more in line with what would be expected. It is interesting to note that the $SO_4^=$ in the concrete gypsum (R10 and R92/3) formed by *Thiobacillus* gives identical ratios. The elementary sulphur (R10A) associated with the gypsum (R10) shows a higher ratio closer to the sulphide (R92/5). The secondary sulphates also have low ratios and, as in the case of the Sydney sewer, a similar ratio to the sulphate in solution in the sewage itself.

This could indicate two possibilities:

(a) That during the formation of sulphate by sulphur oxidising bacteria, through the oxidation of H_2S and S, an enrichment of ^{34}S occurs in the sulphate. This may therefore indicate that elementary sulphur is first formed from H_2S, possibly abiologically, and that this sulphur is then oxidised to $SO_4^=$.

(b) That the sulphate in domestic sewage is derived in a large measure from that formed by bacterial oxidation, and that sulphate reduction is less important than proteolytic decomposition in the formation of sulphides.

MUD AND SEA WATER FROM MILFORD SOUND (A FJORD), NEW ZEALAND

The samples were collected from Milford Sound, a fjord in south-west New Zealand. Although the sediments were highly reducing, generally having a redox potential (Eh) $< O$, sulphide could not be detected in the overlying waters, which contained traces of oxygen.

The mud was collected with a Petersen grab, and hence represents the upper 6 inches only. The water samples were collected close to the bottom above the mud.

The results given in table 13 show that the isotope ratios for the sea water sulphate fall very close to the average of 21·76 given by Ault (1959). The differences from Ault's average may in part be due to the apparent differences between Ault's standard and the New Zealand standard, and partly to the influence of intermixing with fresh water, since the area in which this restricted basin lies has an annual rainfall of 270 inches and a very high run-off rate into the fjord. The influence of fresh water on R240/1 may also be reflected in the somewhat lower sulphate content for this sample.

The sulphate in the sediments show a consistently higher ^{32}S content than that in the sea water. This cannot be explained on the basis of sulphate reduction alone since in that case there would be an enrichment of ^{34}S. Here again, as in other natural environments a number of isotopic fractionation steps may be involved. In the first step sea water sulphate is reduced by bacteria to form a ^{32}S enriched sulphide. The ^{34}S enriched sulphate remaining from this reduction is largely removed from the environment by sea water movements. It would then appear that a re-oxidation of the ^{32}S enriched sulphide occurs in the mud, with the formation of a sulphate enriched in ^{32}S. This sulphate is diluted with the slightly enriched ^{34}S sulphate now remaining from the first reduction to give sulphate slightly enriched in ^{32}S with respect to sea water. This process could at the surface, where a sufficiently high oxygen tension is pres s assumption, an increase with depth down a sediment profile sho uce sulphate correspondingly increasing in ^{34}S content. This suggestion of sulphate formation by hydrogen sulphide oxidation is also reflected in both a higher oxidation-reduction potential as well as a higher iso pe ratio in sample R240/5.

TABLE 13– Isotope Fractionation in Estuarine Mud and Water of Milford Sound (a Fjord)

Station No.	Lab. No.	Sample Description	$SO_4^=$		Acid Volatile $S^=$			Combustible $S^=$		
			%	$^{32}S/^{34}S$	%	$^{32}S/^{34}S$	$\delta\,^{34}S$ Enrichment (‰)	%	$^{32}S/^{34}S$	$\delta\,^{34}S$ Enrichment (‰)
H	R240/1	Bottom sea water .. .. $SO_4^=$ only form of sulphur ..	·234	21·80	–	–	–	–	–	–
A	R240/2	Bottom sea water .. .. $SO_4^=$ only form of sulphur	·268	21·75	–	–	–	–	–	–
A	R240/3	Surface mud .. .. Σ S = ·218% (dry wt) Eh = −100mV, pH = 7·20 $^{32}S/^{34}S$ = 22·47	·280	21·86	·080	22·92	−46·2	·041	22·78	−40·4
G	R240/5	Surface mud .. .. Σ S = ·219% (dry wt) Eh = +15mV, pH = 7·10 $^{32}S/^{34}S$ = 22·39	·262	21·99	·061	22·83	−36·8	·069	22·86	−38·0

The isotope ratios for both the acid volatile and combustible sulphides (probably of the pyrite type as well as perhaps some other case metal sulphides) shows as expected enrichment in ^{32}S. The highest $\delta\ ^{34}S$ depletion shown in table 13 is 46·2 ‰ for the acid volatile sulphide of sample R240/3 with respect to the sulphate in this sample. This ^{34}S depletion falls close to the average value of 46 ‰ found by Feely and Kulp (1957) for sample of sulphides and sulphates from the salt domes of Louisiana and Texas. Since bacterial sulphate reduction in this fjord is still very active (Mr T. M. Skerman, N.Z. Oceanographic Institute, pers. comm.), and no other reasonable explanation can be offered for the formation of such high concentrations of sulphides, we must conclude that fractionations of 46·2 ‰ (or somewhat higher if based on the $^{32}S/^{34}S$ ratio of $SO_4^=$ in sea water) can be attained by such biological systems, under suitable conditions.

Discussion

The results presented above should suffice to illustrate that as yet the stage of knowledge in the field of sulphur isotope biogeochemistry is not far advanced. The processes that cause the fractionation of the isotopes are numerous, as are the valence states of sulphur. The most detailed explanations offered in this paper are based on data from laboratory experiments on bacterial reduction of sulphate, which leads to the formation of hydrogen sulphide enriched in ^{32}S.

Few positive results have as yet been published on work with other biological systems that are responsible for the transformations of sulphur compounds. The fact that such transformations occur in nature is evident from the descriptions of the various environments studied in this paper. Although the process of bacterial sulphate reduction is of paramount importance in the sulphur cycle, and ranks amongst the most important geochemical processes, the other steps in this cycle must not be disregarded. Until these have been studied, the apparently anomalous results cannot be explained. Such anomalies are present not only in this work but throughout the literature dealing with sulphur isotope ratios of sedimentary deposits.

The work on geothermal exhalations has suffered from the same uncertainties, as has that on sedimentary and biogenic deposits. Only preliminary studies by Sakai (1957), and Sakai and Magosawa (1958), Rafter *et al.* (1958), (1960), and Ault (1959) have been reported in the literature, in which an attempt has been made to separate the different sulphur components in the gas-liquid phases. Even in these studies the environmental history of many of the samples studied after deposition was not adequately known. In a previous paper (Rafter *et al.* 1960), an attempt was made to show that once emissions reach the atmosphere they can be subjected not only to inorganic oxidation-reduction reactions, but also to specialised biological processes. One must therefore note carefully the environmental conditions of the immediate area from which a particular specimen is sampled, in order to account for the variables that may have influenced fractionations during and after deposition.

In the present study an attempt has been made to relate isotope variations with the sulphur ecology (sulphuretum) of a particular environment. We have tried to utilise results obtained from planned laboratory experiments to interpret some natural environments. Even though much was known about the origin of the samples studied, and in nearly all instances the samples were being influenced by present-day factors which could be observed and measured, the explanations offered are in many respects inadequate. This work illustrates the hazards of conducting studies on unrelated samples from ancient geological deposits.

We feel justified in drawing attention to the pitfalls involved in this type of study, since they have rarely been discussed in the literature. The *potential* values of these studies have, on the other hand, been given considerable attention by Thode, Jensen, Ault, and others mentioned in this paper. It is evident that once the basic factors influencing fractionation have been studied in greater detail, and the pitfalls understood, such isotope studies will yield information of considerable importance in elucidating biogeochemical processes in nature. The wide range of environmental conditions studied in this article should serve to indicate how generally applicable these types of investigations are.

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